UNIVERSITY OF EDINBURGH

STUDIES OF THE MOLECULAR CONFIGURATION OF POLYSACCHARIDES WITH SPECIAL REFERENCE TO THE CORRELATION OF PHYSICAL AND ORGANIC METHODS OF INVESTIGATION.

by

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CONTENTS.

Page

General Introduction 1

PART I. Experimental Methods of Determining Molecular Configuration.

Introduction 3

Section 1. Isothermal Distillation 5
Section 2. Osmometry 17
Section 3. Viscometry 78
Section 4. Ebulliometry 83

PART II. Results of the Determination of the Molecular Weight of Polysaccharides

Section 1. Studies on Inulin .. 89
Section 2. Studies on Hemicelluloses and Short Chain Glucosans .. 100
1. The Hemicelluloses .. 100
2. Lichenin & Isolichenin 111
3. The Laminarins 115
4. Other Short-Chain Glucosans 120
Section 3. Studies on the Components of Starch
Introduction 123
1. The Nature of the Granular Structure . 125
2. Fractionation and Size of the Starch Components 139

Summary 150

References 152

GENERAL INTRODUCTION.

GENERAL INTRODUCTION

The polysaccharides are an important group of naturally occurring polymers. Organic chemical investigations have shown them to be macromolecules whose structures may vary from simple polymers (e.g. cellulose and the hemicelluloses) to exceedingly complex co-polymers (e.g. the mucopolysaccharides).

Organic chemical investigations usually include an estimation of the number of non-reducing end groups in the molecule. This "end group assay" enables the ratio of the total units in the molecule to the non-reducing end groups to be determined. For a linear polysaccharide molecule this ratio is the average degree of polymerisation. If, however, the molecule is branched, the end group assay only gives the average number of units per unit chain in the molecule, and not the relationship between this value and the molecular size. Moreover, the technique involved in end group assay becomes increasingly difficult as the average chain length of the molecule increases, and the result may be of limited accuracy, when the average chain length is greater than about 100 units.

In order to characterise completely a polysaccharide molecule the results of the chemical end group assay must be considered along with a physico-chemical measurement of the molecular size. A routine method of determining molecular weight is essential therefore for the elucidation of the molecular structure.

A considerable portion of the work to be described was devoted to the development of suitable physical methods. These methods were then applied to problems including the determination of the molecular size of the hemicelluloses, and the components of starch; both of which groups of polysaccharides have been studied extensively in this department.

P A R T   I.

EXPERIMENTAL METHODS OF DETERMINING MOLECULAR CONFIGURATION

INTRODUCTION.

Because a polysaccharide consists of molecules of different sizes, a molecular weight determination results in an average value. The type of average obtained depends on the method used.[1]

If the method used depends on a colligative property (e.g. osmotic pressure or vapour pressure) then the result is a "number-average" molecular weight ($\bar{M}n$), defined as:

$$\bar{M}_n = \frac{\sum_i n_i m_i}{\sum n_i}$$

Where n_i is the number of gram moles of molecular weight m_i, and the summation is taken over all values of i.

Methods which depend not only on the concentration, but also on the weight of the particle (e.g. light scattering and sedimentation), give a "weight average" molecular weight ($\bar{M}_w$), defined as:

$$\bar{M}_w = \frac{\sum_i n_i m_i^2}{\sum n_i m_i}$$

The molecular weight, as determined by the chemical end group assay, is a number-average value. For an accurate comparison, therefore, of such results with those from physico-chemical measurements, the latter methods must also give a number average molecular weight. Osmometry, isothermal distillation, and ebulliometry are all methods which give a number average value, and hence are suitable. These methods were, therefore, developed in this work.

However, each of these methods has a limited range of applicability: isothermal distillation and ebulliometry can only measure the molecular weight of polysaccharides in the range 1,000 to 20,000. Results from one method could, however, be used to confirm these from the other. Osmometry can be used for molecules in the molecular weight range 20,000-1,000,000. By suitable selection of the method, the number average molecular weight of most polysaccharides could be determined.

ISOTHERMAL DISTILLATION

Introduction and Theory.

Many polysaccharides (for example hemicelluloses, fructosans, and glucosans) have a number average molecular weight in the range of 1,000 to 20,000. Isothermal distillation is one method which can be used to determine the molecular weight of polymers of this size.

The method depends on the activity of a solution being less than that of the pure solvent. If a solution is ideal and dilute this difference in activity equals the solute mole fraction, and also equals the difference in vapour pressure between solution and solvent. A solution, in which the solute is associated or dissociated, no longer obeys Raoult's Law, and the activity of such a solution is not ideal, It was assumed here that the solutions studied all obeyed Raoult's Law:

$$\frac{Po - P}{Po} = \frac{n}{n + N} = \frac{\frac{W_2}{M_2}}{\frac{W_1}{M_1} + \frac{W_2}{M_2}}$$

Where Po and P are the vapour pressures of the solvent and solution, $\frac{n}{n+N}$ is the solute mole fraction, W_2 and W_1 are the weights of solute and solvent, and M_2 and M_1 are the molecular weights of solute and solvent.

Now a solution of a polysaccharide with a molecular weight between 1,000 and 20,000 has a detectable vapour pressure lowering, and Puddington[2] has, in fact, described an apparatus in which a sensitive mercury manometer measures directly the vapour pressure of solvent and polymer solution. Such an apparatus was constructed in this Department,[3] but it was found to be impossible to achieve satisfactory working, and the method was discarded.

When a solvent and solution are in a closed system, at constant temperature, with only their vapour phases connected, isothermal distillation takes place. In such a system solvent vapour distils from the solvent into the solution. Both the total amount of solvent distilling, and the rate of distillation are proportional to the vapour pressure lowering of the solution.

Barger[4] in 1903 was the first to observe isothermal distillation of water vapour into drops of aqueous salt solutions hung on microscope cover slips. From this observation Barger developed the "micro" method of isothermal distillation. In this, alternate drops of solutions of known and unknown molarity were drawn into a capillary tube. The drops were observed with a microscope, and adjacent drops which did not change size had the same molarity. Knowing the molarity and weight concentration of a solution, the molecular weight of the solute could be calculated. The micro method has been developed by Niederl[5] and has been used by Caesar[6] to determine the molecular weights of polysaccharide nitrates, but with the exception of Caesar (whose work has not been substantiated), these techniques are applicable only to solutes with low molecular weights.

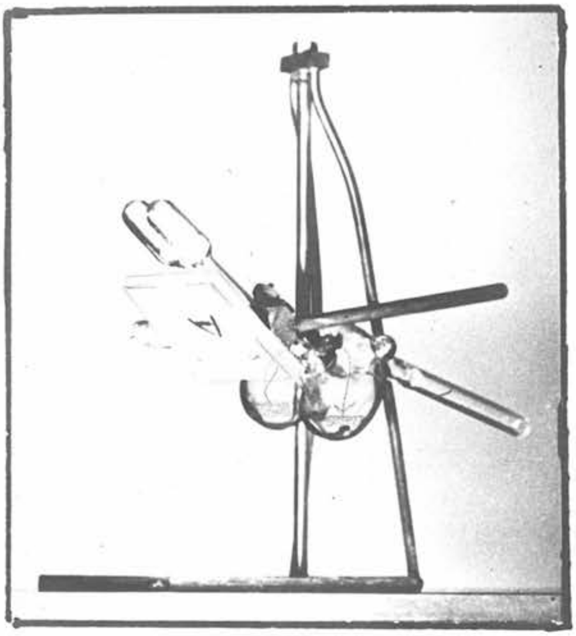
A "macro" method of isothermal distillation, in which solutions of known and unknown molarity were placed in an evacuated dessicator and the vapour allowed to distil until equilibrium was achieved, was used by Bousfield[7] in 1918. In order to calculate the vapour pressure lowering of the solution, the amount of vapour transferred was determined gravimetrically. This method has been reported as applicable for solutions of polymers with molecular weights of 20,000.[8]

Schwarz[9] and Signer[10] have also described a macro method measuring the equilibrium of isothermal distillation. Their apparatus has been adapted by a number of workers including Gee,[11] Clark,[12] White,[13] Childs[14] and Parette.[15]

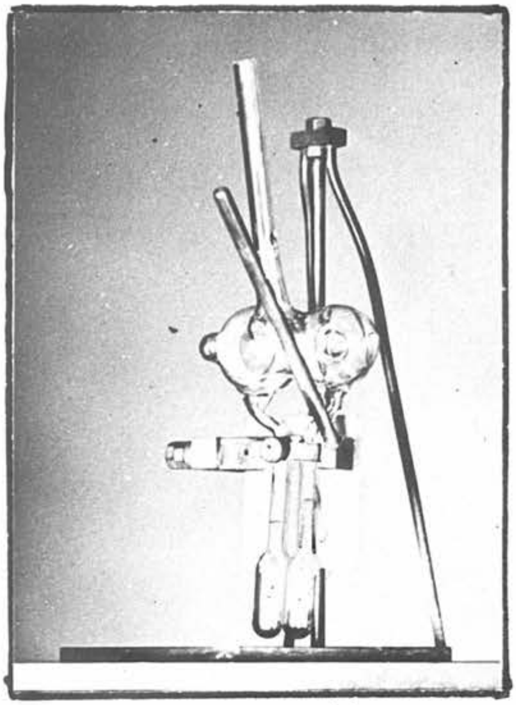
Gee,[11] in 1940, introduced a dynamic method of observing isothermal distillation. Instead of measuring the total amount of vapour transferred between solutions of different vapour pressure, he measured the rate of distillation of vapour from pure solvent into the solution of unknown molarity. This rate is proportional to the vapour pressure lowering of the solution, and the molarity of the solution can be calculated. Gee's dynamic method allowed measurements to be made much more rapidly; and it was this method which was adopted here, for measurements on polysaccharides in the molecular

FIG.1

Isothermal Distillation

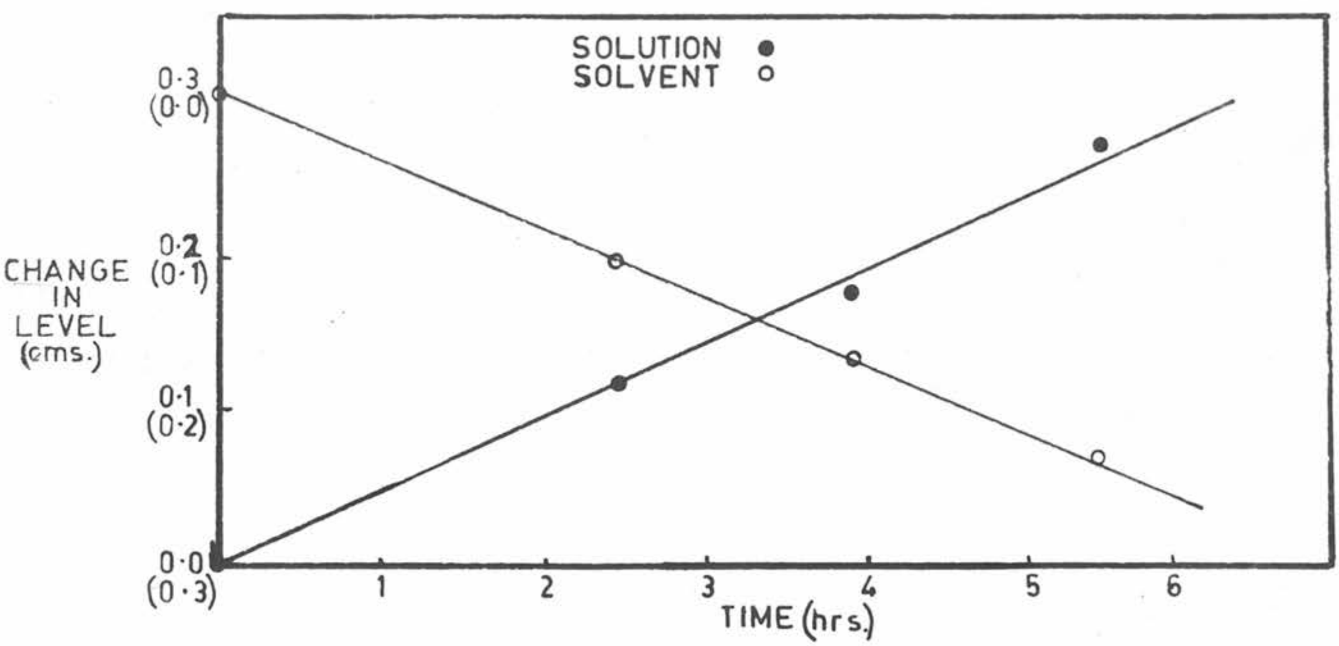


Position (a)



Position (b)

Apparatus



Calibration of Apparatus A
Triolein in Benzene ($N=10\,430\times10^{-3}$)

weight range of 1,000 to 20,000.

Apparatus.

The apparatus consisted of two 3-4 cm. diameter, glass bulbs, joined by a short "U" tube 2·5 cm. in diameter. Perpendicular to the plane of the glass bulbs and "U" tube were attached two measuring tubes (Veridia precision bored tubing) to which were sealed glass bulbs 2 cm. in diameter. There was a reference mark on each tube, just above the bulb. A filling tube was attached, perpendicular to the "U" tube, on the side opposite the measuring tubes. The apparatus was held, on an adjustable stand, in the thermostat bath. The apparatus is shown in Fig. 1.

In apparatus A the diameter of the measuring capillaries was 4 mm.; in apparatus B it was 3 mm., in order to give a more sensitive apparatus.

An attempt was made to modify apparatus A to avoid sealing off the apparatus under vacuum, and to eliminate the consequent, frequent replacement of the filling tube. A 2 mm. bore vacuum tap was sealed to a B10 cone, fitting the corresponding socket sealed to the filling tube. Under vacuum, and in the absence of solvent, the apparatus was leak-tight. When benzene was present, however, water leaked in from the thermostat bath, and the apparatus had to be discarded.

Procedure.

Suitable volumes (ca. 3·5 ml.) of solution and solvent were introduced into the measuring tubes, with the apparatus in position (b), through a length of polythene tubing pushed down the capillary tube and into the mouth of the measuring capillary.

The solution and solvent were degassed in the apparatus, by freezing in liquid air, evacuating, and then warming up in vacuo. The procedure was repeated twice, and, after freezing and evacuating again, the filling tube was sealed off. The apparatus was placed on the adjustable stand in position (a) and transferred to the thermostat bath.

The temperature control of the thermostat bath was very important. If the two bulbs, filled with benzene at 25 C, had a small temperature differential (ΔT °C), then the vapour pressure difference ($^{\Delta P}/_{P}$), derived from the P against T graph for benzene, is $\frac{\Delta P}{P} = 0{\cdot}046\,\Delta T$. The experiment requires $\frac{\Delta P}{P}$ due to temperature differences, to be $< 10^{-4}$, hence ΔT must be less than 0·0022 °C. The thermostat was in fact accurate to 0·001 °C.

After two hours, when the apparatus had attained temperature equilibrium, it was returned to position (b), and the solvent and solution were allowed to drain into their measuring capillaries. Since errors due to incomplete drainage are very large, a standard procedure of allowing half-an-hour's drainage before measuring the levels in the capillaries was adopted. After half-an-hour, any movement of the liquid levels could be attributed to distillation not drainage. The readings, after two hours equilibration and half-an-hour's drainage, were taken as the initial condition for the determination.

The rate of fall of level in the solvent capillary, and the rate of increase of level in the solution capillary were determined by measuring the heights of liquid above the reference marks on the respective capillaries, with a cathetometer reading to 0·001 cm. The time between readings was chosen such that this change in level was about 0·5 mm.

The graph of change in level with time was found to be a straight line (see Fig. 1). The slope of the graph is the rate of distillation, which is proportional to the solute mole fraction. Since the graph is a straight line, the initial distillation takes place at a constant rate.

Hence Rate x K = $\frac{Po-P}{Po}$, where K is an apparatus constant.

$$\text{Rate} \times K = \frac{\frac{W_2}{M_2}}{\frac{W_1}{M_1} + \frac{W_2}{M_2}}$$

This relationship, in agreement with Gee, has been found to hold, down to solute mole fractions of 1×10^{-8} M. The value of K can be determined by measuring the rate of distillation for solutions with known solute mole fractions.

Calibration

Apparatus A was calibrated using benzene as the solvent, triolein (mol. wt. 885·4), raffinose undeca-acetate (mol. wt. 966), and cellotriose undeca-acetate (mol. wt. 966) were used as the standard solutes. K was determined at several solute mole fractions with each solute, and the values of K were calculated for both the change in solvent level and the change in solution level.

As a preliminary experiment, solvent was placed in both sides of the apparatus. With benzene, no movement of the levels was detectable, with chloroform, however, large changes were observed. On this score, chloroform was rejected as a solvent, except for the measurement of the molecular weight of polymers less than 2,000. When carbon tetrachloride was tested, in a like manner, no movement of the levels could be detected. The anomalous distillation of the chloroform was attributed to photochemical decomposition of the chloroform.

Apparatus B was therefore calibrated with carbon tetrachloride as a solvent, and triolein as the standard solute. Since cellotriose undeca-acetate and raffinose undeca-acetate are insoluble in carbon tetrachloride, and since no other substance of high purity and with a known molecular weight about 1,000, was available, the value of K for triolein in carbon tetrachloride in Apparatus B could not be checked with a second standard solute.

Table 1

Calibration of Apparatus A.

Solvent	Solute	Solute Mole fraction x 10^{-4}	Apparatus Constant K x 10^{-3} Solution level	Solvent level
Benzene	Triolein	10·310	2·1	2·2
		2·100	2·7	2·7
		1·756	2·7	2·9
		1·751	3·1	2·9
		0·586	2·3	2·5
Benzene	Raffinose undeca-acetate	13·420	2·5	2·3
		9·343	2·8	2·9
Benzene	Cellotriose undeca-acetate	10·470	2·2	2·4
		9·343	2·5	2·5
		4·880	2·4	2·0
	Average Value of K		2·5	2·5

Calibration of Apparatus B.

Solvent	Solute	Solute Mole fraction x 10^{-4}	Solution level	Solvent level
Chloroform	Triolein	15·430	1·3	1·3
		8·064	0·8	0·8
		5·410	1·1	1·1
		3·704	0·8	0·9
		3·674	0·7	0·8
	Average Value of K		0·9	1·0
Carbon tetrachloride	Triolein	6·613	1·0	1·1
		5·793	1·3	1·3
		2·360	1·3	1·2
		0·875	0·9	1·0
		0·967	1·4	1·5
	Average Value of K		1·2	1·2

Estimation of the Reliability of the Method.

Two experiments were carried out to check the reliability of the results obtained from isothermal distillation.

The first was to determine the molecular weight of a solute at different concentrations. This was done in two cases, a) with a methylated xylan from wheat straw, and b) with a petrol ether precipitated fraction of methylated laminarin; both polysaccharides dissolved in benzene.

The results are shown in Table 2 below.

Table 2

a) Methylated xylan from wheat straw:

Concentration (g./100 ml.)	Time (hrs)	Time (mins.)	Change in solution level (mm.)	Change in solvent level (mm.)
1·7	0	0	0·00	0·00
	4	30	0·15	0·27
	23	50	1·23	1·11
	31	10	1·43	1·54
	47	25	2·30	2·33
	53	0	2·55	2·49
	72	5	3·52	3·64
	77	0	3·73	3·66

$\bar{M}_n$ = 11,800

Concentration (g./100 ml.)	Time (hrs)	Time (mins.)	Change in solution level (mm.)	Change in solvent level (mm.)
0·6	0	0	0·00	0·00
	23	50	0·52	0·42
	48	0	1·05	0·94
	72	5	1·47	1·45
	120	0	2·61	2·42
	144	0	3·03	3·06
	169	0	3·68	3·67
	192	0	4·22	4·25

$\bar{M}_n$ = 12,000

Table 2

b) Petrol-ether precipitated, methylated Laminarin:

Concentration (g./100 ml.)	Time (hrs.)	(mins.)	Change in solution level (mm.)	Change in solvent level (mm.)
0·6	0	0	0·00	0·00
	16	5	0·17	0·56
	46	50	0·99	1·61
	65	5	1·53	2·11
	112	5	2·83	3·38
	137	35	3·46	4·14

$\bar{M}_n$ = 11,900

Concentration (g./100 ml.)	Time (hrs.)	(mins.)	Change in solution level (mm.)	Change in solvent level (mm.)
0·4	0	0	0·00	0·00
	18	0	0·39	0·14
	41	30	0·58	0·41
	66	40	1·09	0·42
	89	15	1·18	0·91
	114	20	1·60	1·12
	163	45	2·09	1·75
	186	0	2·38	1·97

$\bar{M}_n$ = 12,100

Within the experimental error the values of the molecular weights were independent of the concentration. The results also showed the reproduceability which can be expected in a molecular weight determination by isothermal distillation.

The second experiment was to determine the molecular weight of a solute in two different solvents. The molecular weight of methylated lichenin was determined in benzene and in carbon tetrachloride (see Table 3).

Table 3

Methylated Lichenin:

Solvent	Concentration (g./100ml.)	Time (hrs)	Time (mins)	Change in solution level (mm.)	Change in solvent level (mm.)
Benzene	1·4	0	0	0·00	0·00
		18	15	1·69	2·17
		19	35	1·78	1·97
		21	50	1·75	2·16
		39	35	3·27	3·50

$\bar{M}_n = 5{,}000.$

Solvent	Concentration (g./100ml.)	Time (hrs)	Time (mins)	Change in solution level (mm.)	Change in solvent level (mm.)
Carbon Tetrachloride	1·1	0	0	0·00	0·00
		7	0	1·52	1·30
		16	15	3·18	2·67
		20	0	3·80	3·12
		22	0	4·04	3·60
		23	30	4·23	3·82

$\bar{M}_n = 5{,}500$

From these results it was assumed that the value of the molecular weight, as determined by isothermal distillation, was independent of the solvent.

OSMOMETRY.

Introduction and Theory.

The basic equation, derived thermodynamically, for the osmotic pressure (π) of a solution is

$$\pi \bar{V}_o = RT \log_e \frac{p}{p_o}$$

where $\bar{V}_o$ is the partial molar volume of the solvent in the solution, and p and p_o are the vapour pressures of the solution and the pure solvent respectively. This equation is valid over the whole concentration range and reduces to the law of Van's Hoff, i.e.

$$\pi = \frac{cRT}{M}$$

for an ideal dilute solution containing c g. of solute, of molecular weight M, per litre.

If Van't Hoff's law holds then $\frac{\pi}{c}$ should be independent of c. However, it was found experimentally that deviations occurred for polymer solutions, and the empirical expression

$$M = \frac{RT}{(\pi/c)_{\lim. c \to o}}$$

was used to calculate molecular weights from osmotic data. This equation had no theoretical significance until the development of the statistical theory of polymer solutions.

Theory of the Osmotic Pressure of Polymer Solutions.

This theory explains the deviations from Van't Hoff's law of the osmotic pressures of polymer solutions. For an ideal solution

$$\pi \bar{V}_o = RT \log_e \frac{p}{p_o} = -\Delta \bar{G}_o$$

$$\pi \bar{V}_o = T \Delta \bar{S}_o - \Delta \bar{H}_o$$

where $\Delta \bar{G}_o$, $\Delta \bar{H}_o$, and $\Delta \bar{S}_o$ are the change in Gibb's free energy, the heat of dilution, and the entropy of dilution, respectively, occurring when an infinite volume of solution is diluted with 1 g. mole of solvent. The derivation of Van't Hoff's law assumed (1) that the entropy of dilution was ideal (i.e. $\Delta \bar{S}_o = -R \log_e No$, where No = the mole fraction of solvent), and (2) that the heat of dilution was zero. However, for polymer solutions neither of these assumptions is likely to be valid.

The calculation of the entropy of dilution ($\Delta \bar{S}_o$) has been attempted in various ways. Flory and Huggins have calculated the number of ways of arranging long flexible molecules on an array of lattice points using the Boltzmann equation. Each lattice point is occupied by either a solvent molecule, or a polymer segment, and the polymer segments are always adjacent. These calculations, and more recent ones by Flory, have been summarized recently (see "Principles of Polymer Chemistry" by P. J. Flory, Cornell University Press, N.Y., 1953, page 495). All three theoretical expressions simplify at low concentrations to

$$\frac{\pi}{c} = \frac{RT}{M} + Bc$$

where B is a constant. In the limiting case this expression reduces to Van't Hoff's law.

If the heat of dilution ($\Delta \bar{H}_o$) is not zero, it is assumed that the simple relationship for two liquids will hold, viz.

$$\Delta\bar{H}_o = Kv^2 + K'v^3$$

where v is the volume fraction of solute. For low concentrations v is proportional to c, the concentration. Then

$$\frac{\pi}{c} = \frac{RT}{M} + (B-K)c - K'c^2$$

and this again reduces to Van't Hoff's law at infinite dilution.

The calculation of molecular weights from Van't Hoff's law at infinite dilution is, therefore, justified by the present state of the theory of polymer solutions, which also suggests a linear relationship between $\frac{\pi}{c}$ and c at low concentrations.

<u>Calculation of the Constants in the Van't Hoff Equation.</u>

From Van't Hoff's law

$$\pi v = nRT$$

$$= \frac{WRT}{M}$$

where $^{w}/_{v}$ is expressed in grams/litre.

This can be written as

$$M = \frac{cRT}{\pi} \qquad (1)$$

where c is in grams/litre.

When R is expressed in c.g.s. units (i.e. is $8{\cdot}315 \times 10^{7}$ ergs/degree/mole), then π must be in dynes/cm.^{2}, c in gms./cm.^{3} and T in °A.

If the osmotic pressure has been observed in cms. of solvent (h) then

$$\pi = h\,D_o g$$

Where D_o is the density of the solvent at T A, and g is the gravitational constant (981 dynes/cm.^{2}).

If the concentration (C_o) is determined in gms./100 cm.^{3} then

$$C = \frac{C_o}{100}$$

Equation (1) becomes

$$M = \frac{C_oRT}{100\,h\,D_o g}$$

$$\text{or} \quad M = \frac{C_o}{h}\,\frac{RT}{100 D_o g}$$

By substituting the density of the solvent, the appropriate numerical factor to convert $(\frac{C_o}{h})$ to the molecular weight can be calculated.

In the table, this factor has been evaluated for benzene and chloroform at several temperatures.

Table 4

Solvent	Temperature C	Density	$\frac{RT}{100Dog}$ x10^5
Chloroform	20·0	1·48902	1·667
	22·5	1·48427	1·687
	25·0	1·47955	1·707
Benzene	20·0	0·87874	2·827
	22·5	0·87607	2·859
	25·0	0·87340	2·892

Measurement of Osmotic Pressure.

Osmometers have been developed for both aqueous and organic solvents. Many instruments of both types have been described.[16]

The measurement of the low osmotic pressures developed by polymer solutions presents many experimental difficulties. A fundamental one is the preparation of a suitable semi-permeable membrane. The limits of the osmotic method are fixed by (1) the semi-permeability of the membrane for low molecular weights, and (2) the sensitivity of the pressure measurement for high molecular weights.

Several techniques can be used to measure the pressure. These are:

i) the dynamic method, in which the initial pressure head in the osmometer is adjusted to a value which is as close to the anticipated final pressure as possible. After the system has come to thermal equilibrium, the change in pressure is accurately measured as a function of time. The value of the asymptote to this curve is identical with the equilibrium osmotic pressure.

The procedure is repeated, starting with an initial pressure head which is approximately equidistant, but opposite to, the asymptote relative to the first curve.

One half of the sums of the ordinates of the two curves for various values of elapsed time are then computed and plotted, yielding a curve which will converge, more or less rapidly depending on the symmetry of the two experimental curves, to a value which is not significantly different from their mutual asymptote, and which is the equilibrium osmotic pressure.

This method has the advantage of giving results quickly, but has the drawbacks (a) of inaccuracy, due to difficulty in matching the two curves which may not have identical shapes, and (b) of giving no indication of solute permeation.

ii) the static method, in which the development of the osmotic pressure is observed, as a function of time, until equilibrium (see page 31). This method is slower than i), but generally more accurate, and more important it shows whether solute permeation is occurring and enables some correction to be made for this.

iii) another dynamic method, in which an external pressure is applied to the solution, and the rate of movement of the solvent level is plotted against the applied pressure. This graph cuts the applied pressure axis at the osmotic pressure.

The Osmometry of Polysaccharide Solutions.

The application of osmometry to solutions of high molecular weight polysaccharides has been adequately reviewed recently.

a) For starch by Kerr.[17]

b) For cellulose by Ott and Spurlin.[18]

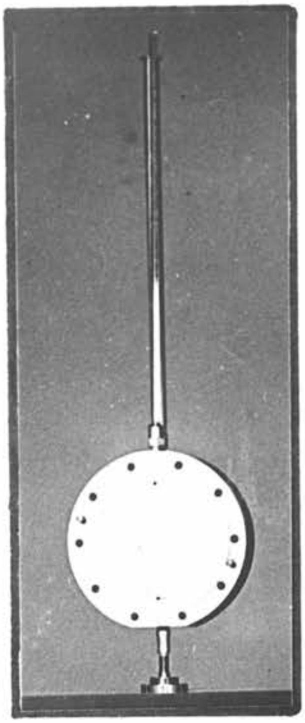
c) For the other polysaccharides by Greenwood.[19]

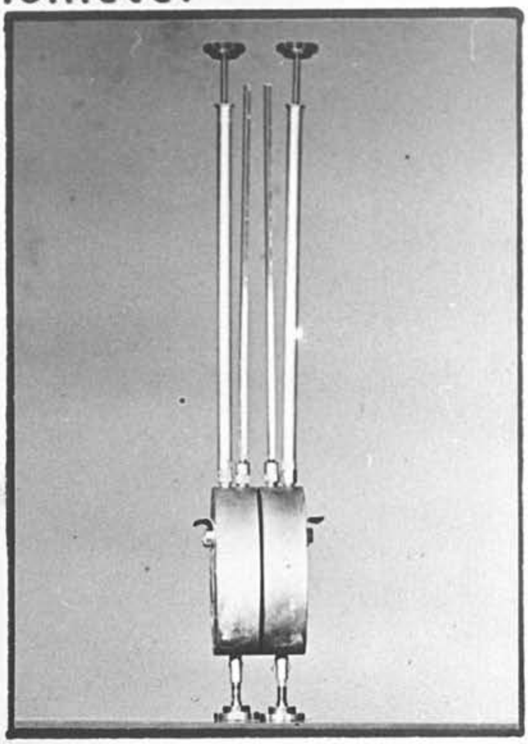
In all instances, instruments capable of measuring small pressures with high precision were required.

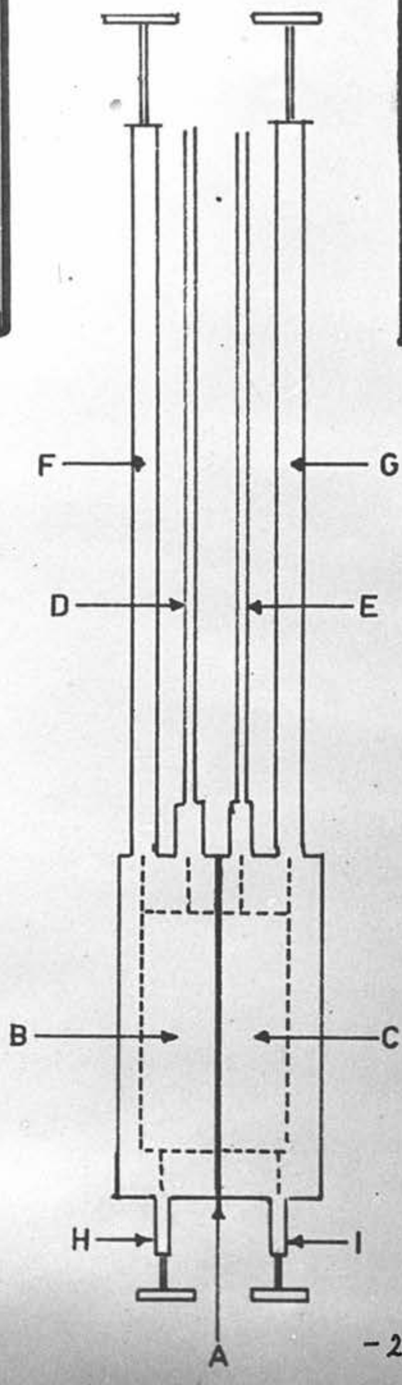
The osmometers used in these investigations were a Fuoss-Mead type, two modified Zimm-Myerson instruments, and a Gilbert, Graff-Baker, Greenwood instrument, from which a new osmometer was developed. A full description of each is given below.

FIG. 2

Fuoss-Mead Osmometer







2) Apparatus and Procedure.

a) The Fuoss-Mead Osmometer.

Apparatus

This osmometer was first used for polysaccharide solutions in organic solvents, and was afterwards converted for use as an aqueous osmometer.

The apparatus (see Fig. 2) resembled the conventional Fuoss-Mead osmometer,[20] in having the vertical membrane (A) clamped between identical brass blocks (B and C), 16·5 cm. in diameter, and 4·0 cm. thick. Concentric grooves, 0·2 cm. wide and 0·1 cm. deep, were cut in the surfaces of the brass blocks. These grooves, when separated by the membrane, formed the solvent and solution cells. The cells were connected to the measuring capillaries (D and E) by holes drilled through the brass blocks. The measuring capillaries were "Veridia" precision bored tubing, 1 mm. bore, attached by copper/glass seals to brass capillary adapters. These adapters were screwed into the block of the osmometer, with their aluminium foil gaskets.

Each block had a stand pipe and valve (F and G). The brass stand pipe was screwed into the block with aluminium foil gaskets. The valves in the stand pipes were long stemmed, stainless steel, needle valves with fibre seatings. The brass drainage tubes (H and I) had short stemmed, stainless steel, needle valves with lead foil seatings.

In order to fill and empty the osmometer a hole was drilled through each block, from the stand pipe to the drainage tube, with a connection to the bottom of the osmometer cell.

Two refinements were, to have the membrane held taut within concentric rings 12·5 cm. in diameter, and to have an extra clamping surface with a tin foil gasket which isolated the cells from the water in the thermostat bath.

Assembly

The apparatus was assembled by laying block (B) horizontal, and closing the stand pipe and drainage valves. The grooved surface of the block was flooded with solvent, and the membrane, stretched between the concentric brass rings, placed in position. Block (C), with both valves closed, was placed on top of block (B) and the two spacers in block (C) ensured that the two blocks came together identically at each assembly. Ten $\frac{5}{16}$ B.S.F. screws, with washers, were used to clamp the blocks together.

Each block had a clamping surface which had been specially lapped to give a leak-tight fit, with the membrane acting as a gasket between the surfaces. The outer edge of the membrane recess was raised and lapped. A tin foil gasket was clamped between this surface and the lapped surface in block (C). It was this seal which gave the additional protection against leaks.

After removing the drainage valves, the apparatus was placed in a specially constructed thermostat tank, the drainage valves were replaced, as quickly as possible, and solvent added to both cells to prevent the membrane drying out. The osmometer was kept in position in the thermostat tank with four positioning screws in the bottom of the tank. A rubber gasket between the osmometer and the bottom of the tank kept the thermostat tank water-tight.

The thermostatting was adjusted to 22·5°C, and was controlled to $\pm$ 0·01°C. This accuracy was attained by lagging the copper tank with asbestos board, by having an efficient stirrer, and by using a chloroform/mercury regulator, with its expansion bulb close to the two 250 watt spiral heaters. The heaters were activated from a valve (K.T.66) relay.

The osmometer was adapted for aqueous osmometry by having it nickel plated, in particular the surfaces of the cells, and the clamping areas. "Teflon" gaskets were used as seatings for the stand pipes and capillary tubes, and "Teflon" seatings were used in the stand pipe valves and drainage valves. 1·5 mm. precision bored capillaries, cemented with "Araldite" thermosetting cement, into brass adapters were substituted for the 1·0 mm. capillaries.

Procedure for osmometry in organic solvents.

The solvent cell was filled by adding solvent down the stand pipe with the valve open. After rinsing the solvent cell several times, the stand pipe valve was closed and the stand pipe filled with solvent. The valve was then opened slightly and solvent allowed to drain into the cell slowly, to reduce the possibility of forming air bubbles in the cell.

The solution cell was filled, similarly, using the same portion of solution to fill the cell as had been used to wash out the cell. This ensured that the solution in the cell was all at the same concentration. The actual concentration was determined at the end of the osmotic pressure measurement.

During measurements, the solution stand pipe valve was removed, while the solvent stand pipe valve was closed. This facilitated the approach to equilibrium, as only a small volume of solvent, namely the volume of the capillary bore above or below the equilibrium level, had to be transferred. The solution stand pipe being open, meant that any solvent flowing into the solution side caused little change in level, since the change in this case was determined by the stand pipe bore, i.e. 10 mm. This system also minimised any errors due to permeating solvent diluting the solution. Solute permeation effects were magnified by the above system. The solvent volume was 7 ml. and the minimum solution volume was 25 ml.

The evaluation of the "cell constant" was the first measurement to be made, after assembling the osmometer. When the apparatus did not have precision bored capillaries, a variable cell constant was observed, even when a membrane with a large hole in it was present. In such a case the cell constant must have been due to irregularities in the capillaries. The capillaries were replaced with the two 1 mm. bore precision capillaries, cut from the same piece of tubing. The cell constant was determined in the absence of a membrane and found to be zero. This value was unchanged when a membrane was present, and in all cell constant determinations since then the cell constant has been zero.

Each membrane, which was used, was calibrated by measuring the time required to half a given pressure ($t_{\frac{1}{2}}$ value, see page 54), with solvent on both sides of the membrane. The $t_{\frac{1}{2}}$ value gave a qualitative measure of the semipermeability of the membrane, and also an idea of the time an osmotic pressure would take to develop and come to equilibrium.

The pressure could be adjusted, either by adding more solvent into the solvent cell from the stand pipe, or by removing solvent through the drainage valve. The level in the solution side remained unaltered during these manipulations.

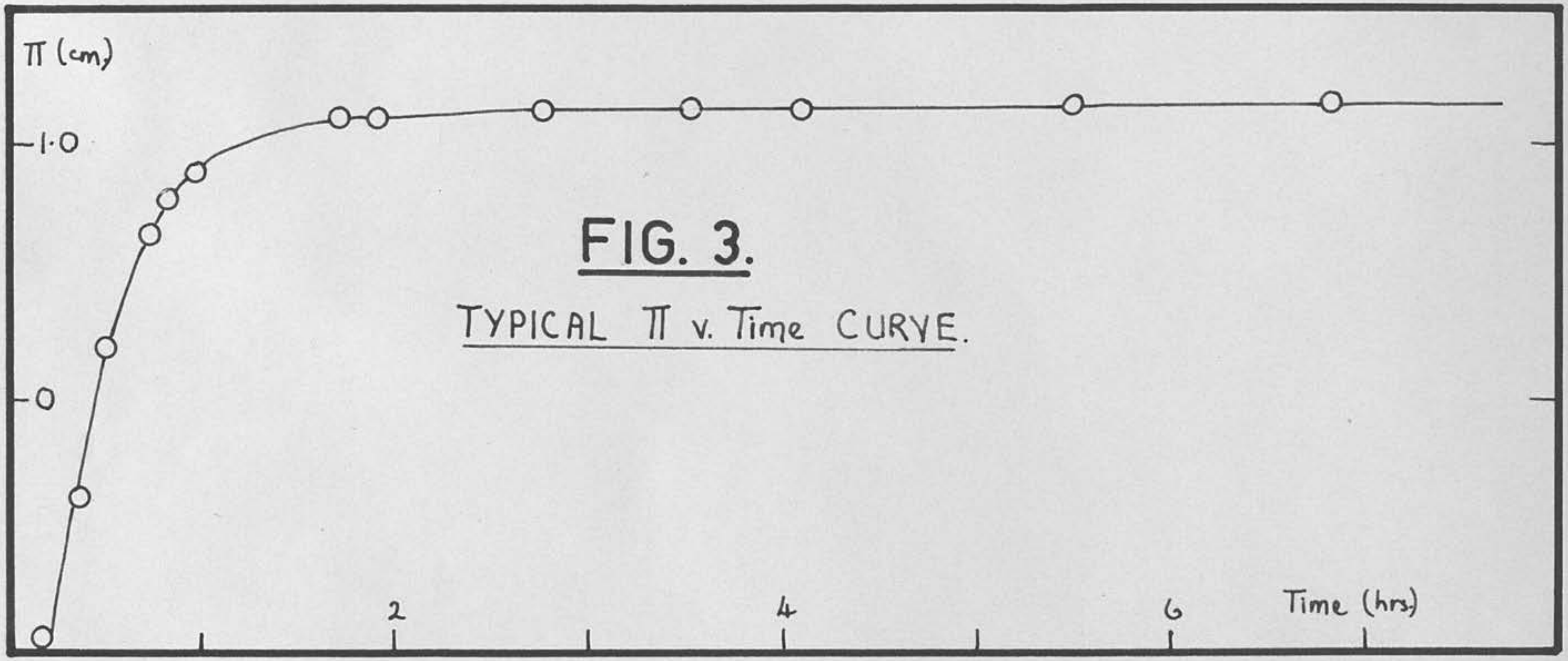


FIG. 3.

TYPICAL Π v. Time CURVE.

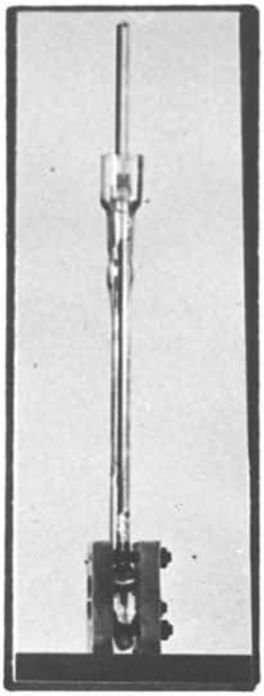
Measurements of the osmotic pressure were made by the static method. A negative pressure was produced, and then the observed pressure was plotted against time until equilibrium had been reached. After equilibrium, the pressure was observed for any decrease, due to solute diffusion through the membrane. The graph for such a measurement is shown in Fig. 3.

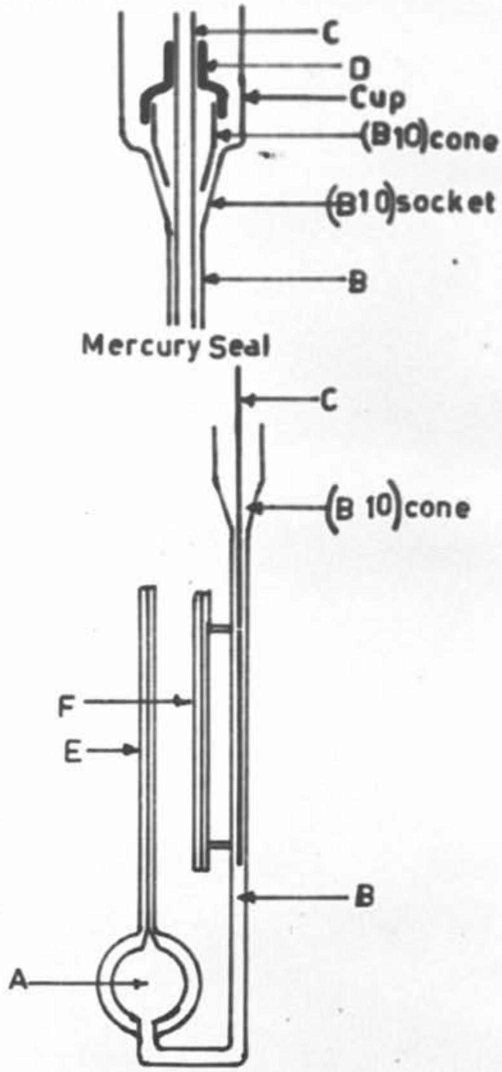
Procedure for aqueous osmometry.

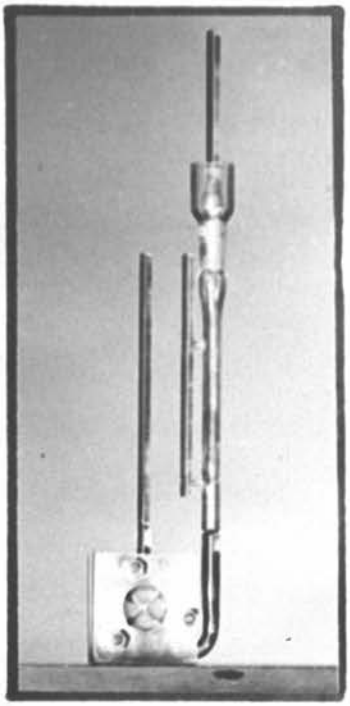
The osmometer was operated in a similar manner. The only additional precaution was to ensure that the capillaries had been carefully degreased. The capillaries were degreased by immersing in hot 1:1 concentrated nitric acid:concentrated sulphuric acid, overnight. They were rinsed, and then immersed for 5 mins. in warm 20% caustic soda solution, rinsed again, and the acid treatment repeated for a further five minutes. The capillaries were finally thoroughly rinsed in distilled water.

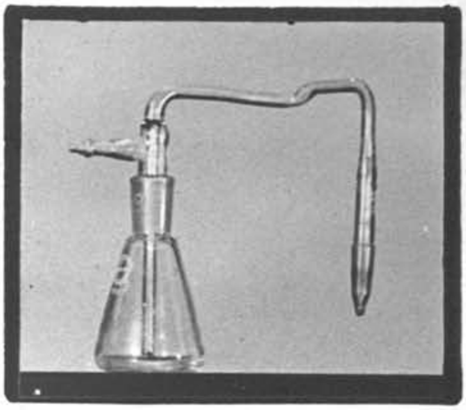
FIG. 4

Zimm-Myerson Osmometer









Filling Device

b) The Zimm-Myerson Osmometer.

Apparatus.

The Zimm-Myerson osmometer, of conventional design,[21] (see Fig. 4) consisted of a thick walled glass cell (A), 22-24 mm. in diameter, and 10 mm. broad. The faces of the side walls were 2-3 mm. thick, and were ground flat. Two vertical membranes were clamped between the walls and the stainless steel plates. The latter had holes 20 mm. in diameter drilled through them, to allow the solvent to reach the outer surfaces of the clamped membranes. The clamping surfaces of the steel plates were machined flat and polished. The membranes acted as gaskets between the plates and the glass cell. Three screws held the plates tight against the walls of the cell.

The filling tube (B) was 20 cm. long and had a ground glass socket (B10), with cup, attached to the top. Fig. 4 shows how the socket and cup were adapted for use as a mercury seal. A glass rod (C) was pushed through a cone (B10) and held within the cone by a piece of polythene tubing (D). The rod fitted into the filling tube where the cone was seated in the socket. When the cup was filled with mercury, this arrangement acted as an efficient seal, and no mercury leaked into the apparatus.

The measuring "capillary (E) was 0·5 mm. "Veridia" precision bored tubing, about 10 cm. long, and attached by a 3 cm. piece of "Pyrex" tubing to an outlet in the top of the solution cell. The reference capillary (F) was "Veridia" tubing of the same diameter, and was attached to the filling tube.

Assembly.

The stainless steel plate, fitted with the screws, was laid horizontal with the screws upwards. A non-wrinkled membrane, 2·5 cm. in diameter (see page 58), was placed in position, centrally, over the stainless steel plate. The membrane was never allowed to become dry during the assembly. The glass osmometer was placed in position and the other membrane laid on top of the upper ground glass clamping surface. The other plate was pushed down on top of the membrane, and screwed into position. Fingertight tension on the screws was sufficient to ensure leak-tight seals. After filling the apparatus with solvent, it was placed in a jar of solvent within the thermostat tank.

The thermostat was a plate glass tank (18" x 12" x 12"). Temperature control was maintained to $\pm$ 0·01°C at 22·5°C by a "Circotherm" unit, which incorporated a heater, thermoregulating thermometer, valve relay, stirrer, and circulating pump.

Procedure.

Filling and emptying the apparatus were accomplished with the aid of the device shown in (Fig. 4). The cone (B10) was fitted into the socket at the top of the filling tube, and when suction was applied at the side tube, the contents of the solution cell were drawn into the 50 ml. flask. By carefully blowing into the side tube, the contents of the flask could be transferred to the osmometer, without any air bubbles being trapped in the filling tube.

The apparatus was filled with solution by adding and removing solution from the apparatus into the flask several times. This ensured adequate mixing of the solution with the traces of solvent left in the osmometer. At the end of the osmotic pressure determination, the solution was drawn into a dry 50 ml. flask, and the concentration of a sample of the solution was determined.

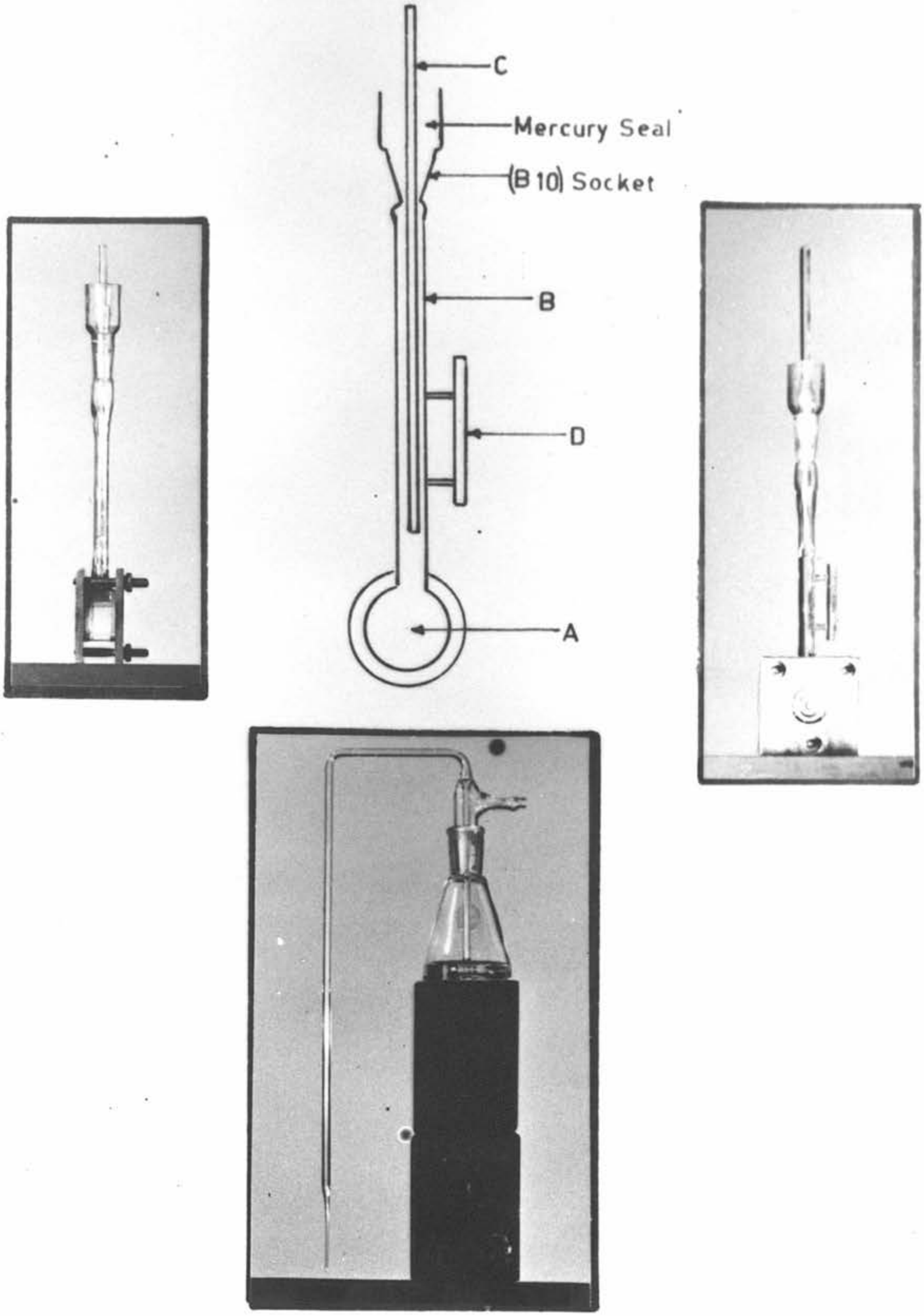
Osmotic pressure measurements were made by the static method described on page.31. The level of solution in the measuring capillary was adjusted to approximately the expected value, by moving the rod (C) up or down in the filling tube, and the change in the solution level was observed.

In this type of osmometer, the membranes were not supported on both sides, as in the Fuoss-Mead osmometer, and they may billow when pressures are exerted on them. A modification to the Zimm-Myerson osmometer has been described,[22] which prevents membrane flap, and which is claimed to give an increased rate of equilibration.

In the osmometer used, the final equilibrium pressure was taken as the mean of several observed values, as no steady pressures were set up. This fact reduced the accuracy of the osmometer, so that only the higher osmotic pressures could be measured satisfactorily.

FIG. 5

Zimm-Myerson Osmometer Modified



Filling Device

c) The Modified Zimm-Myerson Osmometer.

Apparatus.

A simplified, all glass, Zimm-Myerson type of osmometer was constructed, in which only the filling tube was connected to the solution cell. The measuring capillary was held in position inside the filling tube.

The solution cell (A) was a thick walled glass tube, 22-24 mm. in diameter, 10 mm. broad, and 2-3 mm. thick. Brass plates, machined and polished flat, held the two vertical membranes in position, as in the conventional Zimm-Myerson osmometer (see page 33). Small holes were drilled in the plates, to allow the solvent access to the membrane. The filling tube (B) was 13 cm. long, and 5 mm. wide. At the top of (B) a cone (B10) with cup was used as a mercury seal, as before (see Fig. 4). The rod held within the cone was the measuring capillary (C), a piece of precision bored tubing, 0·5 mm. bore, and 15 cm. long. The reference capillary (D), also a piece of 0·5 mm. precision bored tubing, was attached to the filling tube.

Assembly.

The apparatus was assembled in the same way as the conventional Zimm-Myerson osmometer (see page 34).

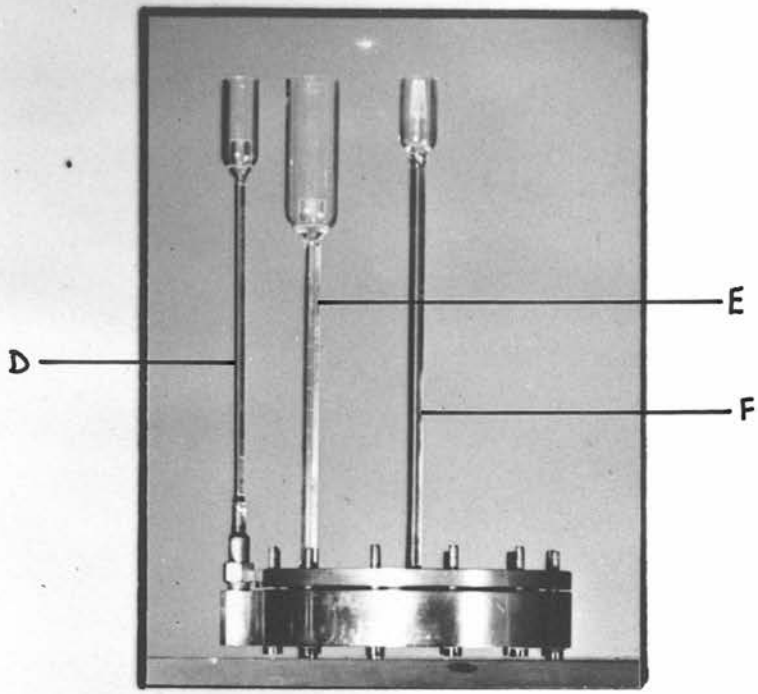
Procedure.

A device, shown in Fig. 5 , was used to fill and empty the apparatus. By sucking, or blowing at the side tube, the solution could be removed from, and inserted into, the apparatus.

Measurements were made by the static method, with the same provisos as with the conventional osmometer. The solution level could be adjusted by moving the measuring capillary up or down through the mercury seal.

FIG. 6

Gilbert, Graff-Baker, Greenwood Osmometer



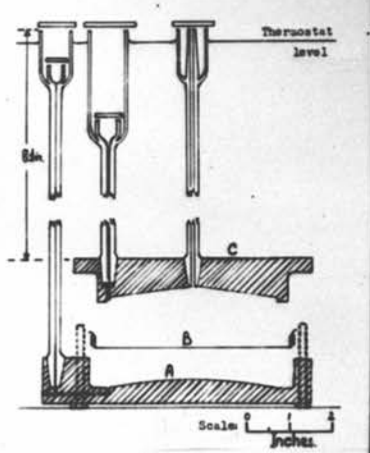


FIGURE 1

The Osmometer.

Vertical section showing (A) the lower half, (B) the membrane in its rings, and (C) the upper half of the cell.

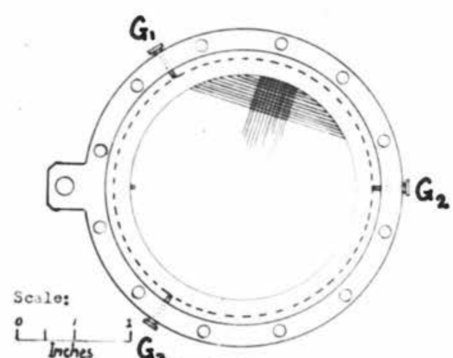


FIGURE 2

Base of Osmometer.

View from above showing grooved membrane support and position of membrane rings (dotted line).

d) The Gilbert, Graff-Baker, Greenwood Osmometer.

Apparatus.

Gilbert, Graff-Baker, and Greenwood have described an osmometer,[23] which was designed for accuracy rather than for ease of manipulation. An osmometer of this type (kindly loaned to us by Dr. G. A. Gilbert) was used for the determination of the molecular weight of the fractionated components from starch. These products, because of their high molecular weight, required an osmometer which would be capable of measuring, accurately, pressures in the order of 0·1 mm.

Accuracy was dependent on very sensitive thermostatting, and a rigid membrane. The rate of approach to osmotic pressure equilibrium was increased by having a large membrane area, and a narrow solvent capillary.

Both cells (A and C) (see Fig. 6) of the osmometer were constructed from brass blocks, which had been nickel plated. The membrane (B), which had been prepared while stretched between two concentric brass rings, 12 cm. in diameter, was clamped horizontally between the upper and lower blocks. Twelve stainless steel nuts held the two blocks together. The clamping surfaces of the blocks were 6 mm. wide, and held the membrane rigid over a domed support, which was 10 cm. in diameter, and had a radius of curvature of 25 cm. To allow solvent access to the lower surface of the membrane, the surface of the dome was grooved. These grooves were of right angle section, 0·2 mm. deep and 1·0 mm. apart.

The lower solvent cell of the osmometer was connected to a "Veridia" precision bored capillary (D) (0·4 mm. bore) by a 1/32" hole drilled in the lower block. The upper solution cell, which was conical in shape, was connected to two capillaries: a measuring capillary (E), and a filling tube (F) both of precision bored tubing (1·0 mm. and 2·0 mm. bore respectively). Cones, which fitted into sockets in the upper osmometer block, were ground on the bottom of each capillary. The cones were cemented in position with magnesium oxychloride cement. A later modification was made to enable the solvent capillary to be changed if necessary. The solvent capillary was cemented, using "Araldite" thermosetting cement, into a nickel plated adapter, which was screwed into the lower block. A thin piece of tin foil was used as a gasket. Each capillary head was provided with a saturater to reduce evaporation. These were small annular cups containing solvent. The capillary heads were covered with small glass plates, grooved on the under surface to ensure atmospheric pressure in the capillaries.

Accurate thermostatting was most important, and the bath used showed no variation in temperature greater than 0·001 $^\circ$C, at 22·5 $^\circ$C, over long periods. The bath was constructed from 1/4" plate glass, 32" long x 17" wide x 12" deep. A 100 watt heater, of rectangular shape (20" x 4"), was suspended parallel to, and 4" from, the back of the bath. A chloroform/mercury regulater was placed in the bath with its expansion bulb near a 1" copper strip, joining the top and bottom sides of the heater. This regulater controlled the heater through a valve relay (Osram PX4). Vigorous agitation of the water in the bath was maintained by a stirrer driven by a 1/8th horse power motor, which also pumped the water through a cotton wool filter. The top of the bath was covered with hard asbestos board.

Liquid levels in the osmometer were measured through the glass walls with a cathetometer reading to 0·001 cm. Condensation on the under side of the capillary cup lids was prevented by gently heating, directly above the lids, with a subsidiary, very low wattage heater.

Assembly.

The lower block was filled with degassed solvent, and the membrane inserted, whilst ensuring that there were no air bubbles trapped underneath it. Three pins (G_1 G_2 G_3) held the membrane central, while the upper cell was lowered into place. The twelve nuts and bolts were then uniformly tightened. Before placing the osmometer in the thermostat bath, the three pins were removed, and the excess solvent drained off.

Procedure.

The contents of the solution cell could be transferred from the cell to the cup at the top of the solution capillary by exerting pressure at the top of the solution capillary (F). After emptying the solution cell, fresh solutions were placed in the capillary cup, mixed by oscillating them between the solution cell and the capillary cup, and then carefully run into the solution cell, taking care to avoid the formation of bubbles. Excess solution was removed from the annular cup, and the saturators on the solvent and solution capillaries were filled with solvent. The glass covers were replaced and the apparatus allowed to come to temperature equilibrium before osmotic pressure measurements were made.

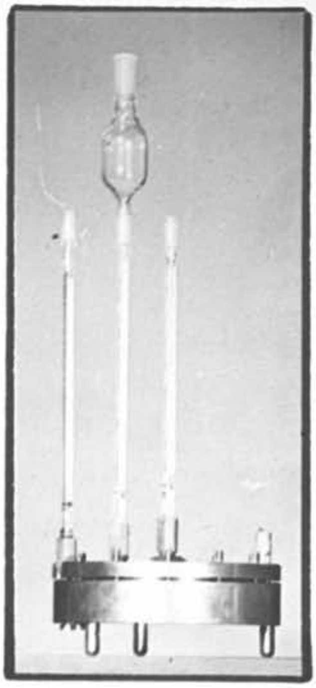
A static method of observing osmotic pressures was used.

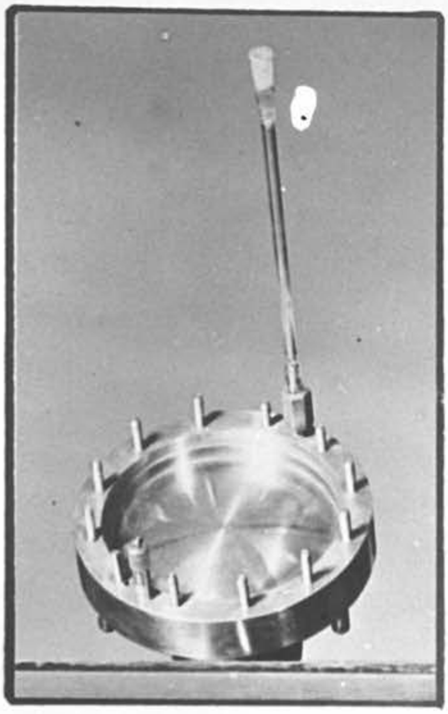
As a preliminary to the measurement of an osmotic pressure, the cell constant of the apparatus was determined. Since the solvent and solution capillaries had different bores, a difference in capillary rise, with solvent in each capillary, was to be expected. This cell constant was about 1·3 cm. when chloroform was the solvent. The cell constant was measured in the absence of a membrane, in order to check that the cell constant was independent of the liquid level in the solvent capillary. With the original 0·2 mm. precision bored capillary, a variation in cell constant was found, and measurements had to be made with the liquid always in the same region in the capillary. This irregularity was probably due to quite small variations in the bore of the solvent capillary. 0·4 mm. precision bored capillary was substituted, and the cell constant was then independent of the liquid level.

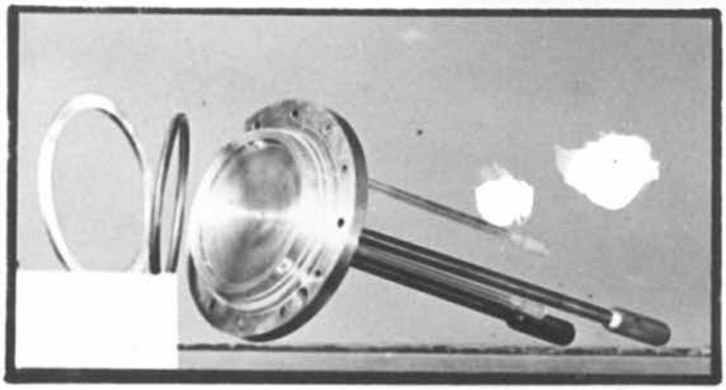
The concentration of the solution was determined at the end of an osmotic pressure measurement by transferring the contents of the solution cell into the capillary cup, and estimating the concentration of solute in a suitable sample.

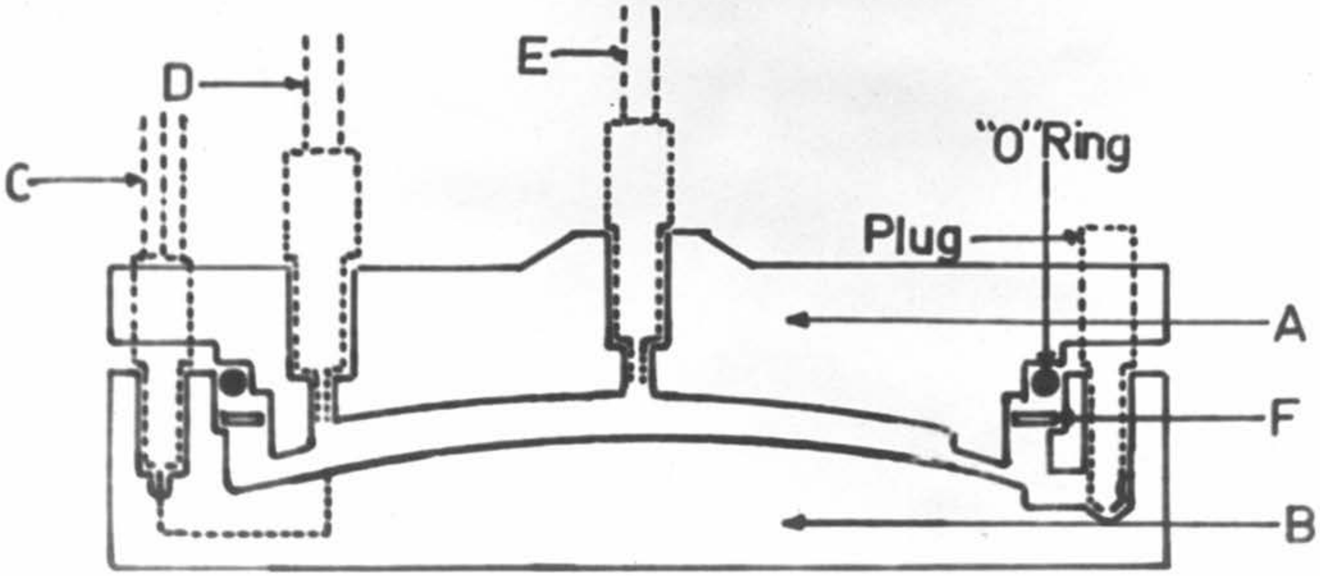
FIG. 7

A New Osmometer









e) A New Osmometer.

A new osmometer, based on the Gilbert, Graff-Baker, Greenwood apparatus, was designed, in collaboration with Mr. C. Graff-Baker. In this apparatus, accuracy was again considered as more important than ease of manipulation. Improvements included (I) an internal seal to ensure that water from the thermostat bath could not reach the membrane, (II) removable capillaries, (III) a more ideally domed support which would give more rigidity to the membrane, and (IV) a complete interconnecting seal for the tops of the capillaries.

Apparatus.

The apparatus consisted of two brass blocks (A and B), 5 3/8" in diameter, which were clamped together with twelve 2 B.A. stainless steel screws. Two holes were tapped in the lower block, one to hold the solvent capillary (C), the other to collect excess solvent during assembly. A small hole (3/16" dia.), through the block, connected the solvent capillary with the lower solvent cell. The clamping surface was ½" wide, and at an angle of 12½ to the horizontal. This angled clamping surface, and a domed membrane support, 3½" in diameter, with a radius of curvature of 8", were the optimum dimensions to ensure the minimum of creasing of the membrane, when it was in the apparatus. These figures were the results of experiments made by Mr. C. Graff-Baker, in which he built several model osmometers to observe directly the creasing of the membrane. There were no sharp edges to the clamping surfaces which might have cut the membrane. The domed support was not grooved, in order to keep the solvent cell volume to a minimum.

Two holes were tapped in the upper block to hold the capillaries (D. and E). The internal seal, mentioned above, was an "Edwards" "O" ring 4 1/16" in diameter, which was held in place, round the upper block, by a brass ring (F), (internal diameter 3 3/4"). This brass ring rested on a ledge in the lower block, and as the upper block was screwed into position the "O" ring sealed the solvent and solution cells from the thermostat water.

Brass capillary adapters screwed into the brass blocks with thin tin foil gaskets. The solvent capillary (C) was cemented into its adapter with thermosetting "Araldite" cement, while glass/metal seals were used to seal the solution capillaries in their adapters.

The glass/metal seals were made by platinising a piece of capillary at 800°C. When the glass had cooled to 150°C, it was dipped in a bath of just-molten lead, to give a thin, strongly adhering, film of lead. The inside of the adapter was tinned with soft solder, and the capillary soldered into the adapter. These seals were leak-tight to a vacuum of 10^{-3}mm. of mercury.

To prevent evaporation of solvent, a ground glass socket (B7) was sealed to the top of each capillary. An interconnecting glass tube ensured that all three capillaries were connected, at the same pressure, and there could be no evaporation.

The apparatus was thermostatted to $\pm$ 0·001°C.

Assembly.

After filling the lower cell with degassed solvent, the membrane, prepared while stretched between two concentric brass rings, 4" in diameter, was placed in position, ensuring that no air bubbles were trapped beneath it. The brass ring (F) was inserted, and it held the membrane taut over the domed support, while the upper block with the "O" ring in position, was lowered onto the other block. The peripheral nuts were then tightened to uniform pressure. During the assembly the drainage hole in the upper block was left open. When the two cells were tightly clamped together, the excess solvent was withdrawn from the hole, and the plug screwed into position. Three legs, 3/4" long, were screwed into the base of the osmometer, and these ensured better temperature control, in that no stagnant water could collect under the osmometer.

Operation.

By applying pressure at the top of capillary (E), the contents of the solution cell could be transferred to a removable cup, with a ground glass cone, which could be attached at the top of capillary (D). With this cup in place, the solution could be added to or removed from the apparatus.

Osmotic pressure measurements were made in a manner identical to that used in the original instrument.

3. General Techniques.

a) Solvents and Solutions.

Chloroform and benzene were the two solvents used in osmometry.

The chloroform was degassed before use, by boiling for a short time, and then cooling in a stoppered flask. Degassed solvent was most essential for the two osmometers with the horizontal membranes, as the formation of gas bubbles in the solvent cell entailed the dismantling of the apparatus.

"Analar" benzene was used without any pretreatment, and gave satisfactory results.

Solutions in chloroform, for the new osmometer and the original, were filtered before insertion into the apparatus. Each solution was filtered several times through a G3 sintered glass filter, and several times through a G4 sintered filter. Using such filtered solutions, no obstructions were formed in the narrow capillaries of the osmometers.

b) Estimation of Concentration.

The concentration of the solutions, used in all the osmometers, was determined at the end of the osmotic pressure measurement. A small sample of solution (ca. 2 ml.) was transferred to a counterpoised weighing bottle, and weighed. 10 ml. of solute precipitant, usually petroleum ether (b.p. 80-100°C), was added to the solution in the weighing bottle. The main bulk of the liquid was then evaporated on an electric hot plate. More precipitant was added, and the evaporation continued to dryness. The weighing bottle and contents were then placed in a vacuum drier at 90°C overnight, after which they were cooled and weighed. This procedure prevented the formation of a "glass" of polymer: the latter has been shown to cause large errors in the estimated concentration.[24,23]

c) Membranes.

The whole success of osmometry depends upon the behaviour of the membrane. This should be, ideally, semipermeability to solute, yet at the same time reasonable permeability to solvent. In practice it is very difficult to achieve such a system.

(i) Theories of Membrane Action.

Many theories exist to explain membrane action. One of these is the "molecular sieve" theory,[25] in which membranes are regarded as porous structures through which solvent molecules are able to permeate. Solute molecules, on the other hand, are too large to move through the pores. Complications arise, however, when direct observations are made of the pores' sizes, and the pore diameters are found to be greater than the diameters of the solute molecules which have been held back by the membrane.[26,27]

Another theory[26,28,29] postulates that adsorption forces, from the surface of the membrane, act across the pores and so reduce the effective diameters to less than the directly observed pore diameters. These adsorption forces cause solvent molecules to become attached to the surface. Then, by a displacement action, the solvent molecules can progress through the membrane, from the region of high, to the region of low, solvent activity. The adsorbed solvent molecules no longer act as a solvent for the solute molecules, which are themselves negatively adsorbed, never becoming adsorbed on the membrane surface.

On this theory, a membrane will be impermeable to solute molecules, as long as the pores of the membrane are not so large that the adsorption forces are not effective right across the pores. In which case, there is a channel within the pore, where there is no adsorption effect and down which both solvent and solute molecules are free to travel.

There is a third theory of membrane action: the solubility theory,[30] which postulates that the membrane will only be permeable to those substances which dissolve in it. This theory is inadequate to explain the action of the inorganic ferrocyanide membranes, which are permeable to organic solvents.

(ii) Types of Membrane used in Osmometry.

Two types of membrane are used in osmometry:

a) the inorganic colloids, e.g. the copper ferrocyanide membranes,

b) the organic colloids, e.g. regenerated cellulose and poly-vinyl-alcohol membranes.

In the work reported here, only the latter type of membrane has been used. The materials included bacterial cellulose, poly-vinyl-alcohol, and gel cellophane (cellophane sheet which has never been allowed to dry).

(iii) Permeability of Membranes.

Membrane permeability was expressed as the "$t_{\frac{1}{2}}$ value." This value was determined by setting a positive pressure in the osmometer, and then plotting pressure against time. This curve was exponential, and the time required for the pressure to decrease by a half was constant. It was this time which was known as the $t_{\frac{1}{2}}$ value, and which was a measure of the relative permeabilities of different membranes, in the same solvent, in the same osmometer.

(iv) Evidence of Pinholes in Membranes.

Immergut, Ranby and Mark[31] have reported a method by which the presence of pinholes in a membrane may be detected. If the solvent diffused homogeneously through the membrane, an exponential decay, with time, of a positive pressure (P), ought to be found

$$P_t = P_o e^{-Dt}$$

If on the other hand the solvent streamed through

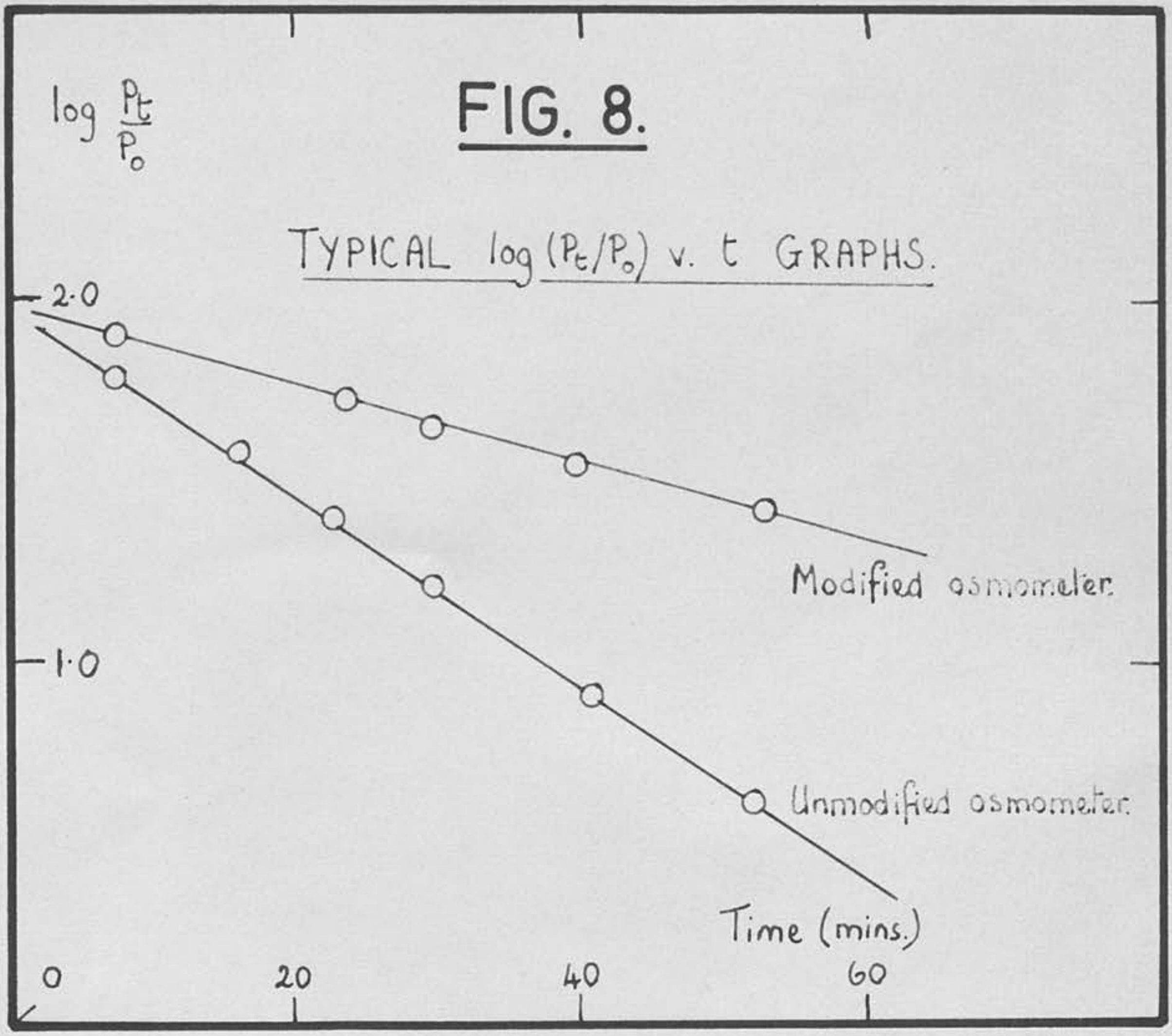


FIG. 8.

TYPICAL $\log (P_t/P_o)$ v. t GRAPHS.

pinholes in the membrane, the decrease in pressure should follow the relation

$$P_t^2 - P_o^2 = - Dt$$

On the basis of this, the graph of log $^{Pt}/P_o$ against t should be a straight line, if there are no pinholes in the membrane.

This procedure was tried with gel cellophane (No.600) membranes which had not been treated with ammonia. Results were derived for membranes in the modified, and unmodified, Zimm-Myerson osmometers. Since both graphs were straight lines (see Fig. 8), the membranes could be assumed to be free from pinholes and suitable for osmometry.

(v) Behaviour of Bacterial Cellulose Membrane.

Bacterial cellulose membranes have the advantage of greater permeability than cellophane membranes, and a consequent more rapid approach to equilibrium.[32] Only one bacterial cellulose membrane was used in this work. This was found to possess a much lower permeability than usual, and it was, therefore, hoped that it would be able to be used to measure smaller molecular weights, without solute permeation occurring. The very low permeability, however, rendered the membrane impractical, due to the long times required for osmotic pressure equilibrium. No other bacterial cellulose membranes were available.

(vi) Behaviour of Poly-vinyl-alcohol membranes.

Poly-vinyl-alcohol membranes[33] (kindly given to us by Dr. H. T. Hookway) were used in the modified Zimm-Myerson osmometer. In view of the unyielding nature of the membranes, gaskets made from "Nylon 265" were usually placed on either side of the membrane. The $t_{\frac{1}{2}}$ value, in some early determinations, was only one minute. A value attributed to pinholes in the membrane. When a second pair of membranes were tried the $t_{\frac{1}{2}}$ value was in the order of 12 hours. This was much too large for the membrane to have any practical value, since equilibrium could not be expected in less than several days.

A new set of membranes were then inserted without the Nylon gaskets, and had a $t_{\frac{1}{2}}$ value of $4\frac{1}{2}$ hours, a value much more suitable for static osmotic pressure measurements. The cell constant for the apparatus, which should have been zero, was observed, in this case, to be a variable value about 0·2 cm. In spite of this a measurement of the molecular weight of a methylated laminarin was attempted. At no time, however, could the osmotic pressure be measured. The pressures varied by centimetres between hourly observations. The irregular results could not be attributed to a major diffusion of solute through the membranes, because the solvent was removed after the osmometer had been in position for three weeks. The solvent was evaporated down to small bulk, but gave no precipitate when thrown into petroleum ether (b.p. 80-100 C), which was known to be a precipitate for methylated laminarin.

The unsatisfactory behaviour of the poly-vinyl-alcohol membranes has prevented any use being made of them in osmometry.

(vii) Behaviour of gel cellophane membranes.

Gel cellophane was stored in distilled water containing thymol as a preservative. After thoroughly washing the membranes with water, they were conditioned to chloroform, or benzene, by the following scheme.

The washed membranes were soaked, successively, for one hour, in 25%, 50%, and 75% acetone/water mixtures. After which, the membranes were stretched between the concentric clamping rings, so giving taut, unwrinkled membranes. The stretched membranes were then soaked for one hour in 100% acetone, and for a further hour in a 50% mixture of acetone and the required solvent. The final treatment in pure solvent lasted for at least two hours. These membranes were found to be the most generally useful of the three tried, and, therefore, all the osmotic pressure measurements were made with them.

(viii) Ammonia Treatment of Gel-Cellophane Membranes.

Flory[34] has described a treatment for gel cellophane membranes, which includes soaking them in 7N ammonia solution at room temperature. When this procedure was tried, it was found that the $t_{\frac{1}{2}}$ value for the ammonia treated membranes was greater than that for an untreated

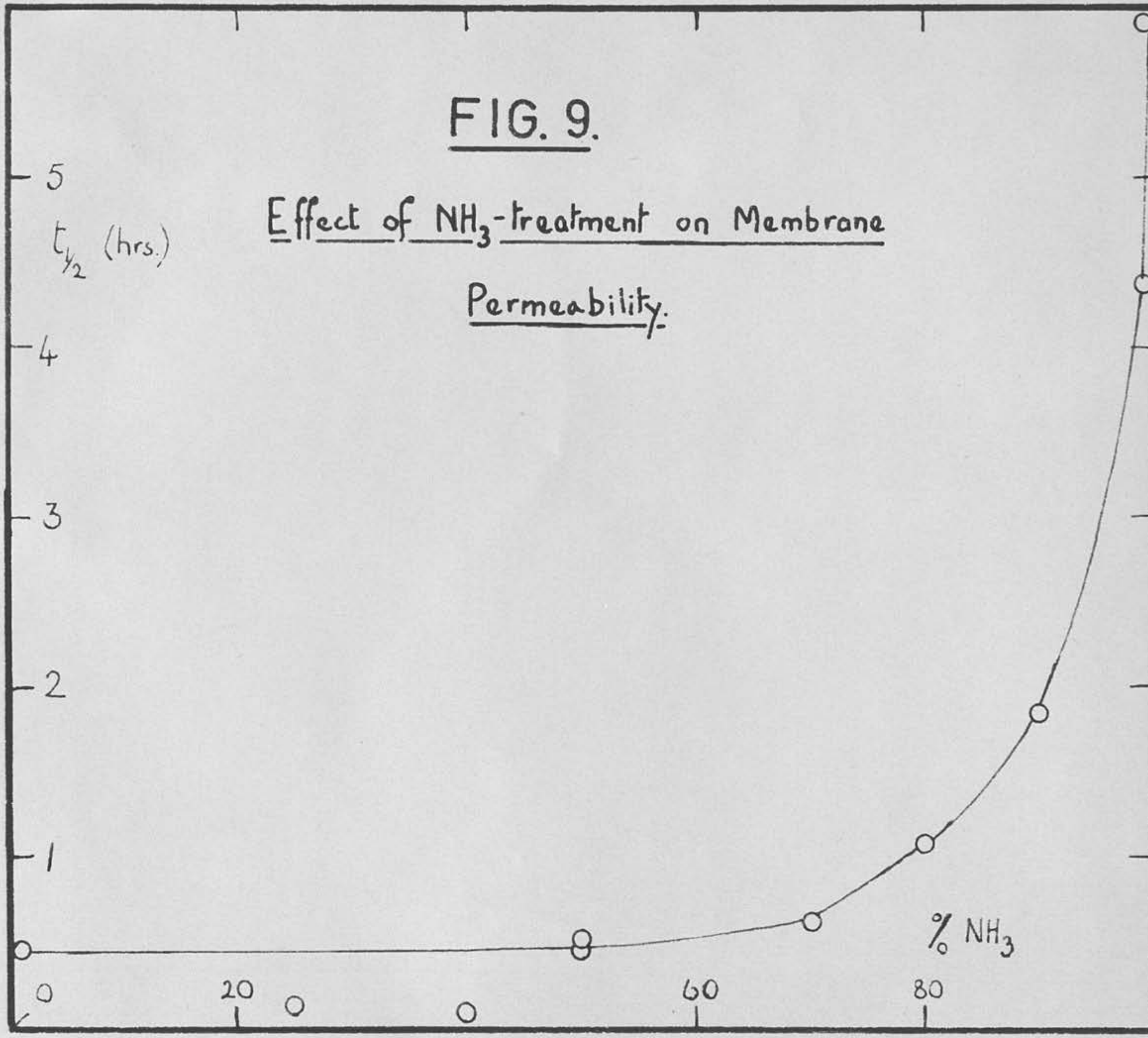


FIG. 9.

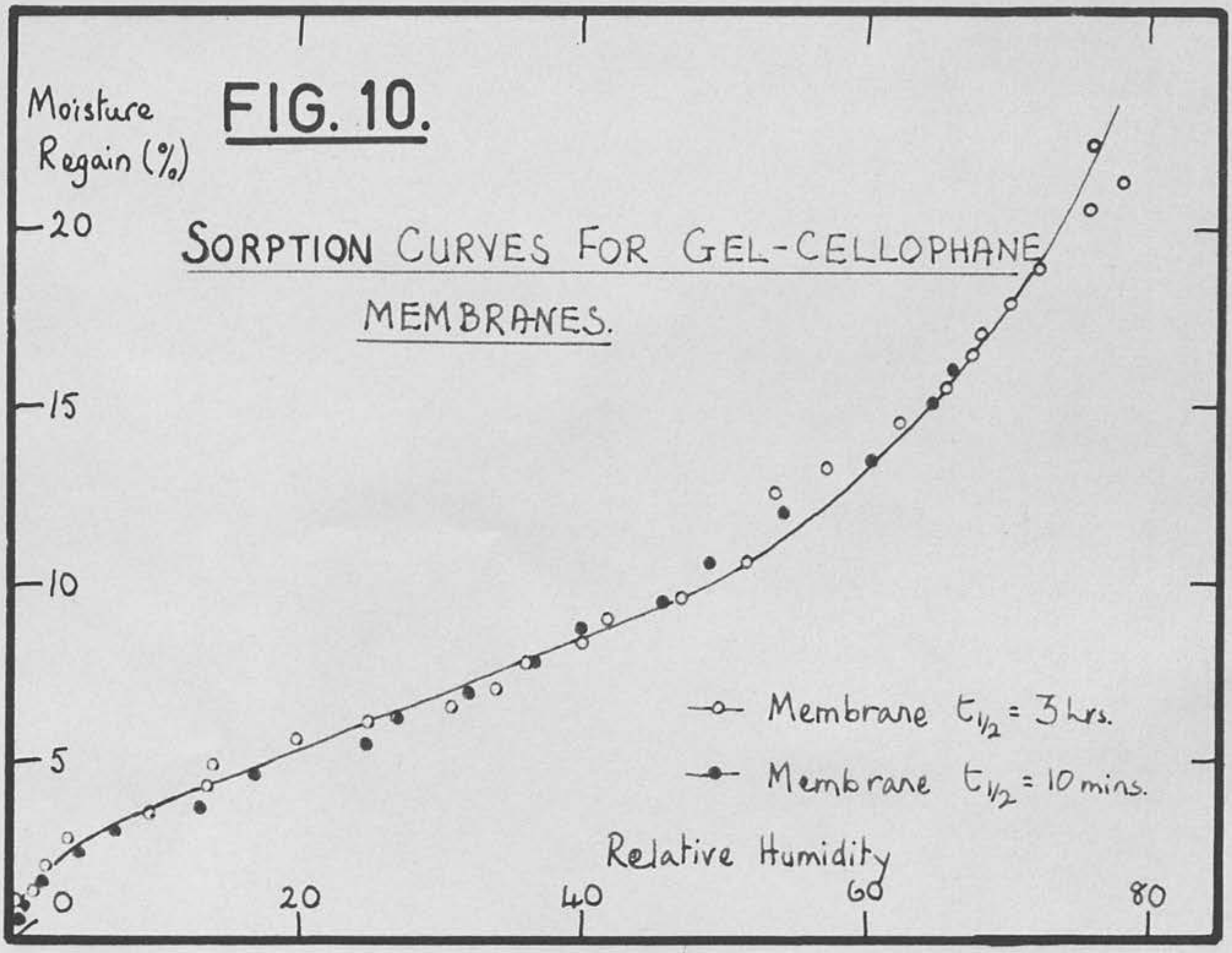
Effect of NH_3-treatment on Membrane Permeability.

membrane (i.e. the membranes were less permeable). This appeared, therefore, to be a method by which the permeability of a membrane could be controlled. A series of membranes were prepared, treated with differing concentrations of ammonia, and their $t_{\frac{1}{2}}$ values determined.

Pieces of gel cellophane (No.600) cut from the same sheet were washed in cold water, then soaked for 10 minutes in hot distilled water. Membranes, in duplicate, were placed in a 25%, 40%, 50%, 70%, 80%, and 100% solutions of 0·88 ammonia in water. The pairs of membranes were thoroughly washed in cold water, conditioned to benzene, and the $t_{\frac{1}{2}}$ value determined. The results are shown in Fig. 9 . From this it can be seen that for concentrations of ammonia up to 70% there was little or no effect on the permeability of the membranes. With higher concentrations, however, the permeability was greatly decreased. Treatment of the membranes with ammonia is, therefore, a method of controlling the permeability of gel-cellophane to an organic solvent.

(ix) Sorption Measurements on Gel Cellophane Membranes.

Information on the permeability of membranes to vapours can be obtained from a study of their sorption isotherms. Two membranes, with vastly different permeabilities as determined by the $t_{\frac{1}{2}}$ value ($t_{\frac{1}{2}}$ values of 3 hours and 10 minutes respectively), were studied in a simple McBain-Baker sorption balance.[35] The sample


Moisture Regain (%)
FIG. 10.
SORPTION CURVES FOR GEL-CELLOPHANE MEMBRANES.
20
15
10
5
0
20
40
60
80
Relative Humidity
Membrane $t_{1/2}$ = 3 hrs.
Membrane $t_{1/2}$ = 10 mins.

was placed on the glass spring (sensitivity 14·42 cm./g.), and the apparatus evacuated. Small quantities of water vapour were admitted, and after equilibrium had been established, the vapour pressure was measured as the difference in mercury levels in a manometer. The moisture regain of the sample was determined by the extension of the spring. Graphs of moisture regain (%), against relative humidity (calculated from the observed vapour pressure and temperature) are shown in Fig. 10. The curves for the two sets of observations were the same, within experimental error. From this experiment there appeared to be no relation between the permeability of a vapour phase, and the permeability of a liquid phase, through cellophane membranes. This only confirms that the action of osmotic membranes depends on the pores, and not the fine structure, of the membrane.

d) The Determination of the Density of Polymer Solutions.

The osmotic pressure, expressed as a solvent height, yet observed as the difference in height of a column of solution and a column of solvent, requires correction for the difference in density between solvent and solution (see page 69). In order to calculate this density correction, the density of the solution must be determined with an accuracy of about 1×10^{-5}.

Preliminary experiments using a specific gravity bottle, and Sprengel-Ostwald pyknometers (equipped with ground glass caps on the side arms) were not particularly successful. The specific gravity bottle had too large a capacity for the small quantities of material available, while the pyknometers were very awkward to fill, and to adjust, so that they contained the same volume of liquid each time.

Apparatus.

The difficulties of filling[36] and adjusting[37] can be overcome by using a self-filling pyknometer, with scales etched on the vertical capillaries. By having the capillary arms of suitable length (at least 12 cm.), and the bore suitably narrow (0·6 mm. precision tubing), the loss of volatile solvent by evaporation can be eliminated.[38]

The apparatus consisted of a 4 ml. bulb, blown on one side of a precision bored capillary "U" tube. 2 cm. of the upper end of the other side arm were bent over to form a hook, for filling the pyknometer, and for hanging the pyknometer in the balance. Each side arm had a reference mark etched on it.

Procedure.

The end of the hooked side was dipped into the liquid, which was drawn into the pyknometer by capillary action, and then the instrument was filled by siphoning. Enough liquid was drawn in, so that the levels in the side arms were above the two reference marks. The apparatus was then transferred to a thermostat tank, accurate to $\pm$ 0·01°C. After half-an-hour in the thermostat, the heights of the liquid levels above the reference marks were measured with a cathetometer (reading to 0·001 cm.). The apparatus was then removed from the thermostat, carefully dried, and weighed on a semimicrobalance to the nearest 0·01 mg. To obtain the weight of liquid, the pyknometer had previously been weighed empty. The volume of the liquid was calculated from a calibration of the apparatus.

<u>Calibration</u>.

The calibration was carried out using benzene of very high purity as the standard liquid. The weights of liquid, for different levels in the capillary arms, were measured. The calculated volumes of benzene were then plotted against the sum of the levels above the reference marks. (See Table 5 and Fig. 11). From a knowledge of the sum of the liquid levels, the volume present in the pyknometer could be calculated.

Table 5

Calibration of the Pyknometer with Benzene at 22·5 C.

Sum of liquid levels cm. (x)	Wt. of Pyknometer + Benzene g.	Wt. of Benzene g.	Vol. of Benzene cc (Y)
1·109	18·42868	3·72874	4·25510
1·300	18·43001	3·73007	4·25661
2·096	18·43562	3·73568	4·26395
2·357	18·43707	3·73713	4·26467
2·980	18·44156	3·74162	4·27111
3·795	18·44702	3·74708	4·27602
4·203	18·44961	3·74967	4·27898
4·815	18·45418	3·75424	4·28420
5·704	18·46041	3·76047	4·29130
6·928	18·46854	3·76860	4·30058

Method of Least Squares.

To calculate the best straight line graph for the above data the method of least squares was used. The values of $\Sigma(x)$, $\Sigma(y)$, $\Sigma(xy)$, and $\Sigma(x^2)$ were calculated. These values were substituted in the simultaneous equations:

$$\Sigma(y) - a_o n - a\,\Sigma(x) = o$$

$$\Sigma(xy) - a_o\Sigma(x) - a\,\Sigma(x^2) = o$$

The equations were solved for a, and a_o, where n is the number of observations made.

Then $Y = a_o + a\,(X)$.

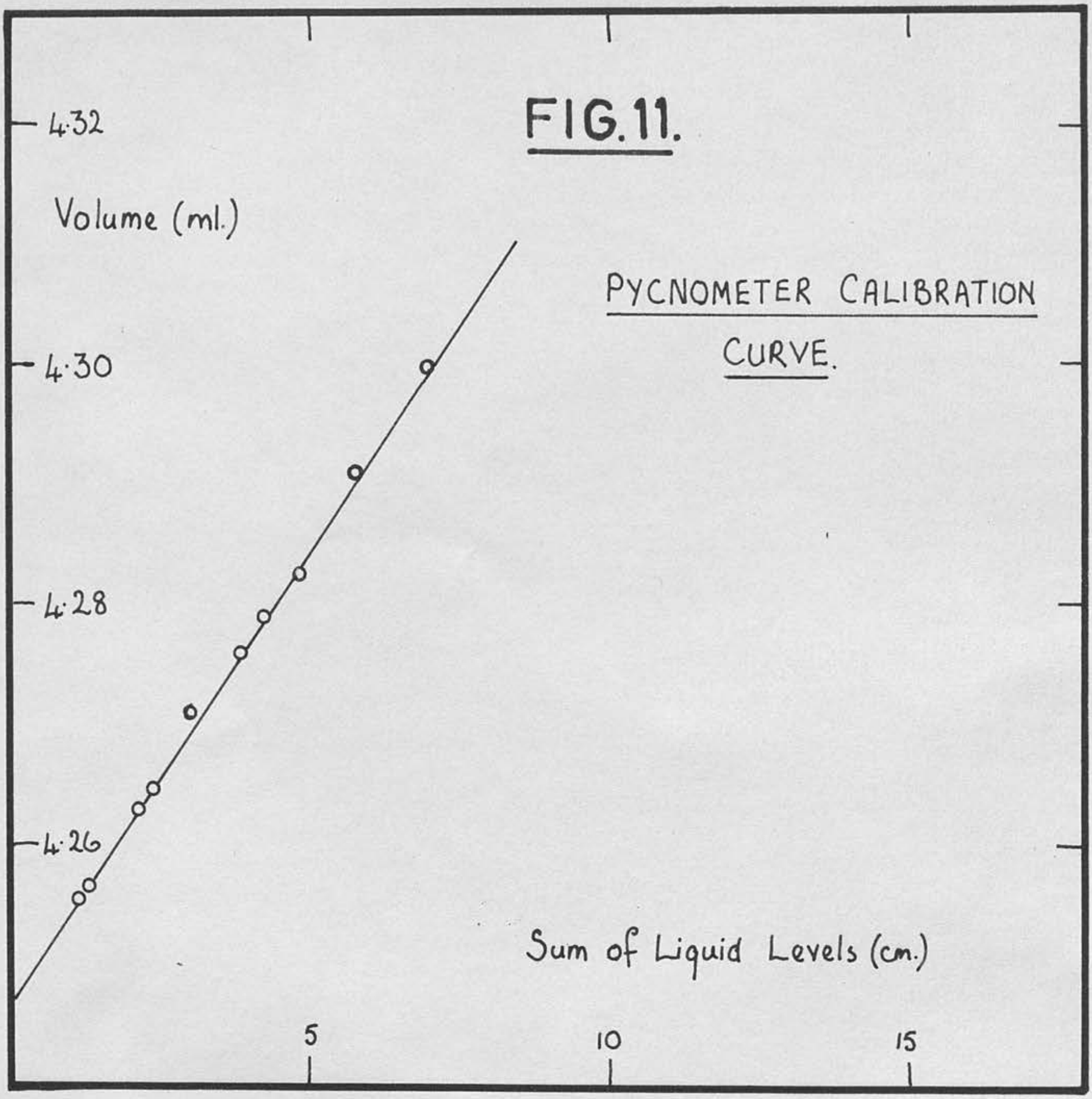


FIG. 11.
Volume (ml.)
PYCNOMETER CALIBRATION CURVE.
4·32
4·30
4·28
4·26
Sum of Liquid Levels (cm.)
5
10
15

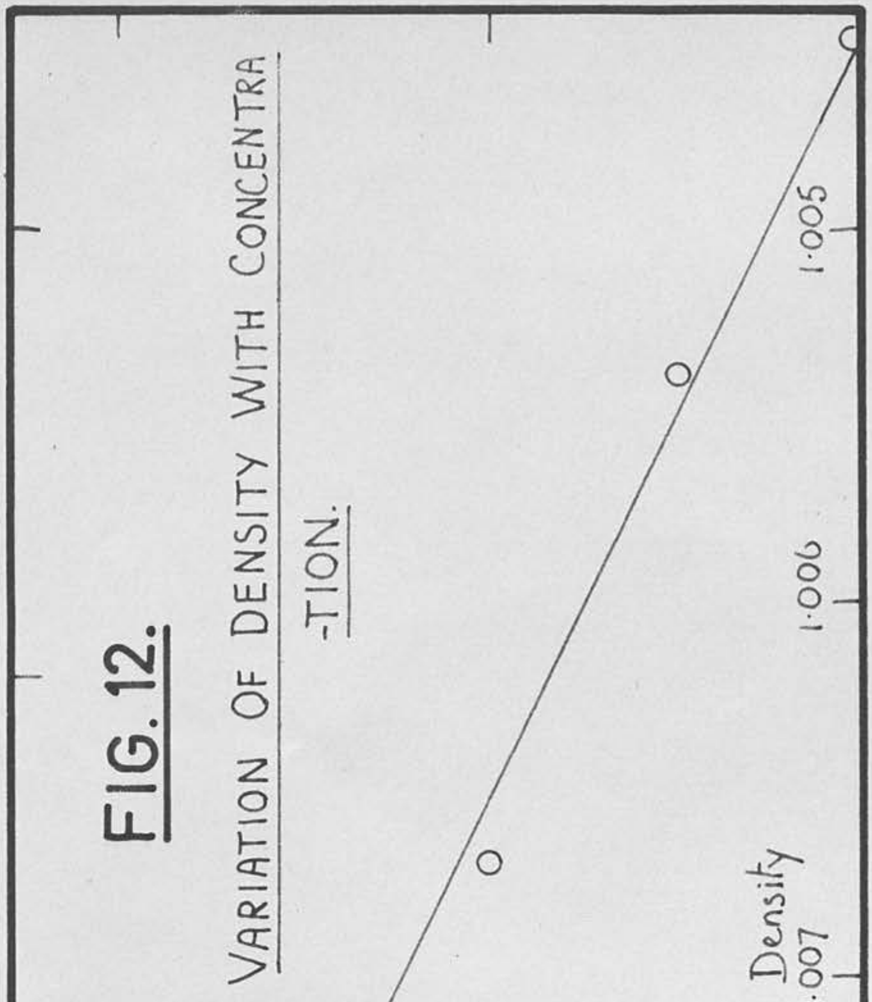


FIG. 12.

VARIATION OF DENSITY WITH CONCENTRA-TION.

For the data from Table 5.

$$\left.\begin{aligned} \sum(x) &= 35\cdot28700 \\ \sum(y) &= 42\cdot74159 \\ \sum(xy) &= 151\cdot07999 \\ \sum(x^2) &= 157\cdot53321 \end{aligned}\right\} \quad \text{Values derived from calculations on an adding machine.}$$

Substituting these values and solving the equations gives

$$Y = 4\cdot24651 + 0\cdot00781\,(x).$$

This line is drawn through the observed points plotted in Fig. 11.

Operation.

The procedure for the determination of the density of a liquid was as in the procedure above, first determining the volume of liquid, and then the weight of that volume. The results for a series of density determinations on solutions of raw gum from Grandifolia in 0·75M NaCl are shown in Table 6 and Fig. 12, as a typical example.

Table 6

Density of Solutions of raw Grandifolia Gum in 0·75M NaCl.

Sum of levels cm.	Vol. of Solution cc.	Wt. of Solution g.	Density of soln. g./cc.	Concn. of soln. g./100 ml.
2·423	4·26543	4·30124	1·00840	1·00
1·784	4·26044	4·29299	1·00764	0·75
1·274	4·25646	4·28501	1·00671	0·50
4·401	4·28088	4·30388	1·00538	0·25
4·306	4·28014	4·29956	1·00448	0·00

4. The Density Correction in Osmotic Pressure Measurements.

Since the osmotic pressure of a solution is expressed as a solvent height, for the purposes of the calculation of the molecular weight, but is measured as the difference in level between a column of solvent and a column of solution, a correction must be applied to account for the different densities of the two liquid columns. The corrected height of the solution column is obtained by multiplying the measured height (less the capillary rise) by the ratio of the density of the solution to the density of the solvent.

The corrected osmotic pressure $\pi_{cor.} = h_s \frac{ds}{do} - h_o$ where h_o and h_s are, respectively, the heights of the solvent and solution columns above the level of a horizontal membrane, and above the centre point of a vertical membrane.[23] d_s and d_o are the respective densities of the solvent and the solution.

The density of a solution is given by

$$d_s = d_o + \frac{c(d-d_o)}{100d} \quad \ldots\ldots (1)$$

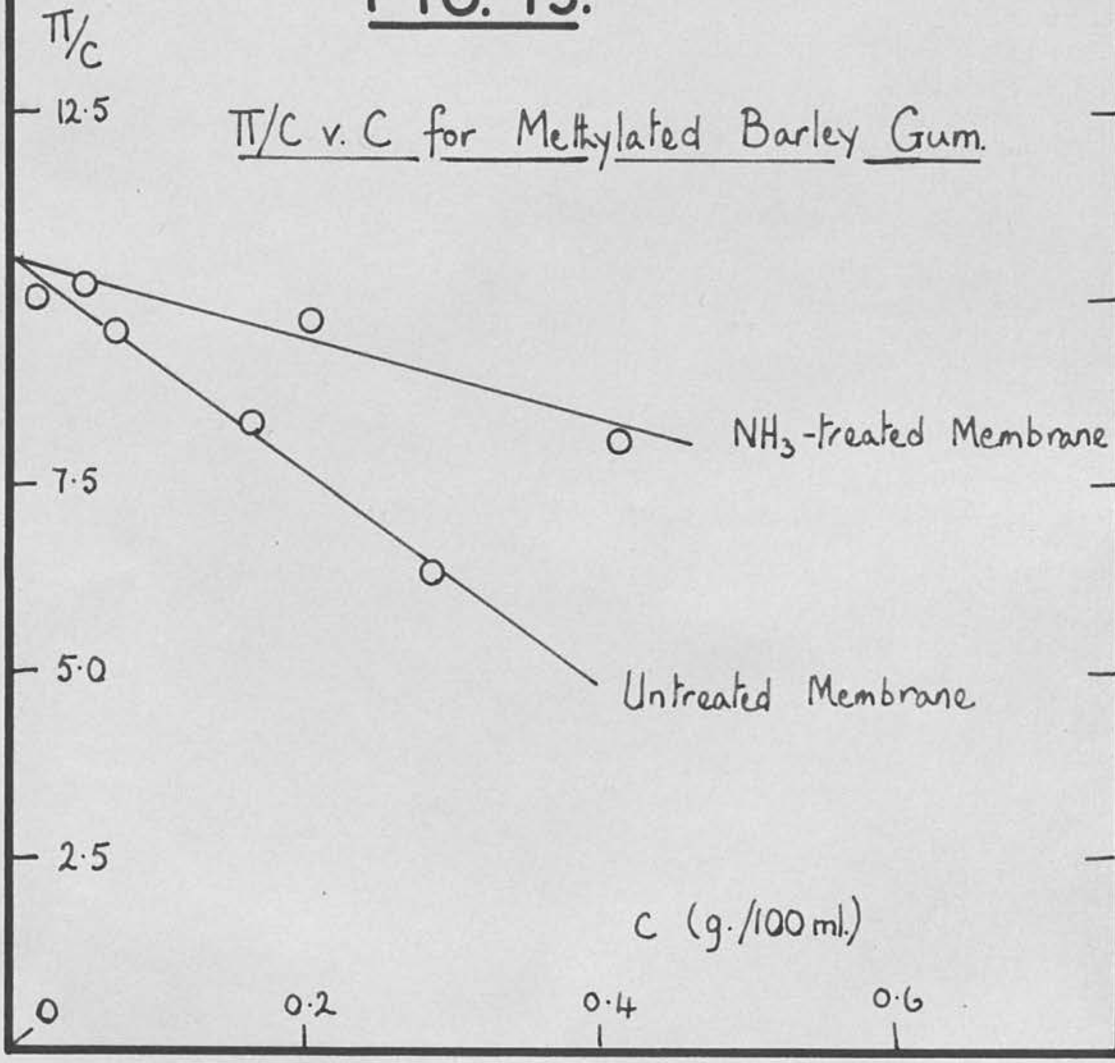
where c is the concentration in g./100 ml. of solution, and d is the density of the solute.

$$\pi_{cor.} = h_s \left[\frac{d_o}{d_o} + \frac{c(d-d_o)}{100d\ d_o}\right] - h_o$$

$$= h_s + \frac{h_s c(d-d_o)}{100d\ d_o} - h_o$$

$$= \pi_{obs} + h_s cK \quad \left(\text{where } K = \frac{d-d_o}{100d\ d_o}\right).$$

FIG. 13.



π/C v. C for Methylated Barley Gum.
π/C
12·5
7·5
5·0
2·5
NH₃-treated Membrane
Untreated Membrane
C (g./100 ml.)
0
0·2
0·4
0·6

In order to calculate K, the density of the solute must be known. By accurately measuring the density of a solution of known concentration the density of the solute can be calculated from equation (1) above. The value of $h_s cK$ can then be evaluated for each concentration and solution height, and so allow the corrected osmotic pressures to be calculated.

e) <u>An Apparent Dependence of $\frac{\pi}{c}$ on the Membrane.</u>

The results of an osmotic pressure determination on methylated barley gum, using a conventional Zimm-Myerson osmometer and No.600 gel cellophane (non ammonia-treated) membranes, are reported on page 121. Later another series of measurements were made on the same sample, in the same osmometer, but using ammonia treated No.600 gel cellophane membranes. These latter results are given in Table 7 below. The graphs of $\frac{\pi}{c}$ against c for the two determinations are shown in Fig.13. It can be seen therefore, that treating the two sets of results independently, the same molecular weight is obtained, but the slopes of the two graphs are different. This phenomenon appears to contradict the results of Menčík.[42] Without carrying out a further series of experiments, no hypothesis can be advanced to account for these results.

Table 7

Methylated barley gum:

Solvent	Concentration (c g./100 ml.)	Osmotic Pressure (π cms. solvent)	$\frac{\pi}{c}$
Benzene	0·416	3·39	8·15
	0·206	2·05	9·94
	0·051	0·52	10·21

5. Estimation of the Accuracy of Measurements.

Whenever a new osmometer was brought into operation for the first time, or a new membrane was inserted in an osmometer, some measure of the efficiency of the new conditions was required. This was accomplished by making an osmotic pressure determination with a standard solute, which had already been thoroughly investigated.

The solute chosen was a broad fraction of polystyrene which had already been the subject of several osmotic pressure investigations.[39,33,40] It was known that the fraction had a low molecular weight "tail," which would diffuse through all except the most selective of membranes. So the rate of decrease of the equilibrium osmotic pressure was also observed to give more information about the efficiency of the membrane.

The original osmotic pressure measurements were made on the sample using the Gilbert, Graff-Baker, and Greenwood osmometer, and in addition one measurement was made with the Fuoss-Mead osmometer. The membranes used were gel cellophane (No.600), untreated with ammonia, and conditioned to chloroform in the usual way. Solute permeation was observed in both osmometers. It was corrected for by extrapolating pressures linearly back to zero time. The consistent results from the two osmometers seemed to be evidence that the correction was adequate.

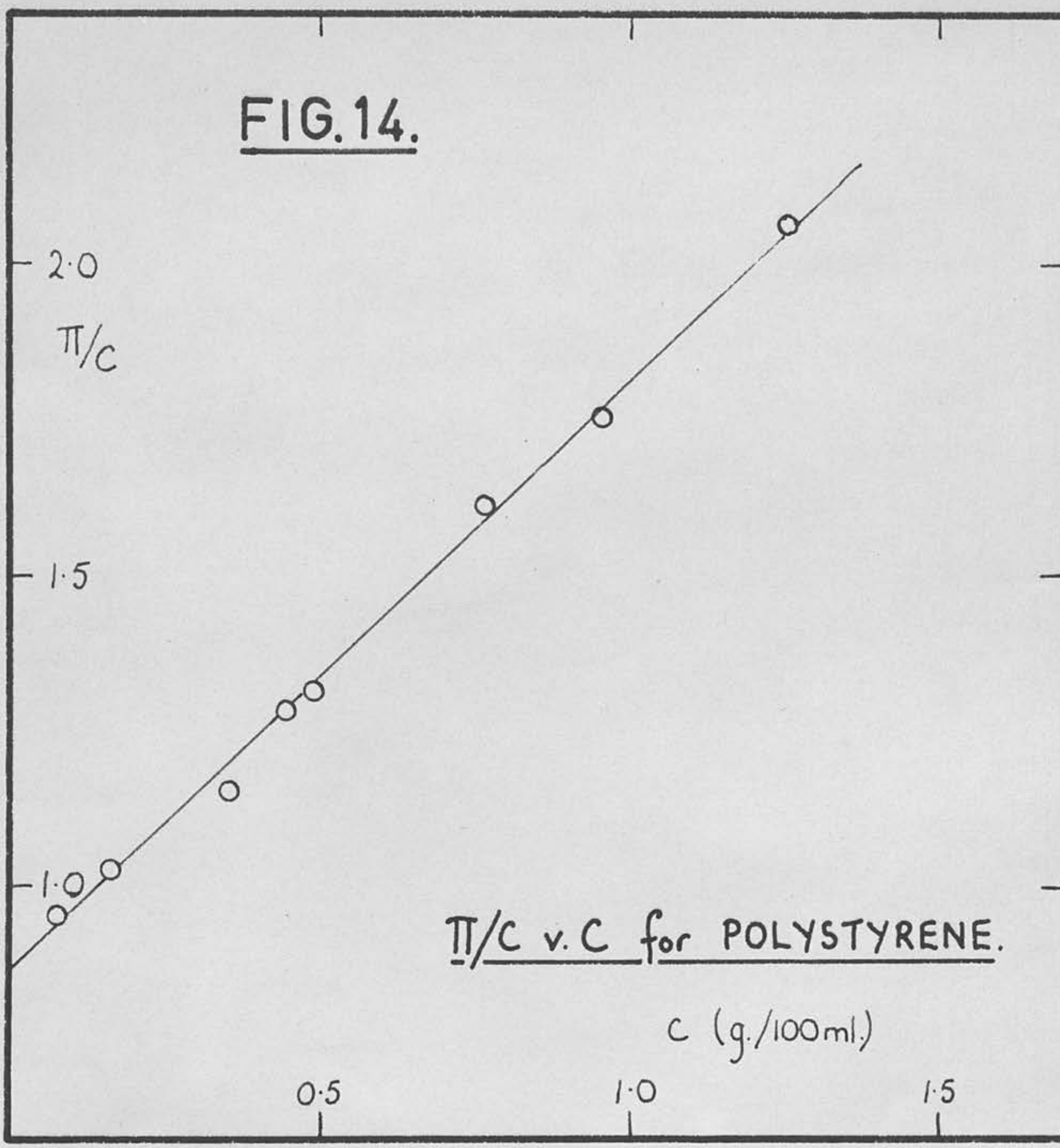


FIG. 14.
2·0
π/c
1·5
1·0
π/c v. c for POLYSTYRENE.
c (g./100 ml.)
0·5
1·0
1·5

The results shown in Table 8 include corrections for the density of the solution (see page 69). The π/c v. C. curve appeared linear (see Fig. 14) and application of the method of least squares showed that the data were best represented by the equation:

$$\pi/c = 0\cdot861 + 0\cdot952\ C$$

Table 8

Osmotic Pressure Measurements on Polystyrene in Chloroform Solution at 22·5 C.

Concentration g./100 ml. (C)	Osmotic Pressure cm. solvent (π)	π/c
1·257	2·596	2·07*
0·956	1·682	1·76
0·766	1·238	1·62
0·487	0·641	1·32
0·443	0·569	1·28
0·358	0·413	1·15
0·270	0·280	1·04
0·165	0·169	1·02
0·079	0·075	0·95

* Measurement in the Fuoss-Mead osmometer.

These figures give a value of 195,000 for the number average molecular weight, which may be compared with 222,500 and 210,000 obtained using denitrated collodiun and a Fuoss-Mead osmometer,[39] and 156,500 using poly-vinyl-alcohol membranes in a Zimm-Myerson instrument.[33]

Sources of discrepancy, other than solute permeation (for which adequate correction may have been made) were 1) membrane dissymetry, 2) solute adsorption on the membrane, and 3) neglect of the appropriate correction for density.

There was no evidence of membrane dissymetry. The effect of adsorption of solute may well vary with the type of membrane used. In these experiments, the effect on the osmotic pressures of any random adsorption of solute on the membrane was eliminated, as all concentrations were measured, in duplicate, after each determination. Proof of the absence of any preferential adsorption of either low, or high, molecular weight solute was difficult to obtain. It was thought that such effects were not occurring as 1) consistent osmotic pressures were observed, independently of the order in which solutions of varying concentrations were measured, and 2) in an additional experiment there was no change in the observed osmotic pressure, when the osmometer was filled, successively, with two solutions of the same concentration (i.e. $\pi = 0{\cdot}420$ and $0{\cdot}419$ cm. chloroform, respectively). Both "selective" and "non selective" adsorption could have influenced the other determinations carried out on this sample.

Density corrections are extremely important when high molecular weight substances are being examined, particularly when comparisons are made between the results from different osmometers: although negligible in the case of the Zimm-Myerson instrument, they are considerable in the Fuoss-Mead type osmometer. For example, if Cleverdon's result of 222,500 were corrected on the basis of an average height of solution column of 20 cm. the value of $\overline{M_n}$ would be 215,000.

The most likely reason for the discrepancy was the low molecular weight "tail" escaping notice in the measurements giving the higher values. Low molecular weight material was certainly present in the sample, as shown in our measurements, although it was not apparent in those of Cleverdon. If this was so, then extrapolations applied for solute permeation must be incorrect. It may also be noted that Staverman[41] believes that this method of correcting for solute diffusion is inadequate.

Sufficient data was, however, available, so that if the osmotic pressure, as measured in any new set of conditions, was in agreement with the results above, then the new conditions of measurement were satisfactory.

VISCOSITY

Introduction and Theory.

Viscosity measurements can be carried out with ease and high accuracy, but the method is not absolute and requires calibration.

In a capillary viscometer, where the flow time (t) of a fixed volume of liquid through a capillary is measured, the viscosity (η) is given by the equation

$$\eta = Kdt - \frac{Bd}{t}$$

where K & B are constants and d is the density of the liquid. B is a kinetic energy correction which can be calculated by making measurements of t with different pure liquids of known viscosity and density. In order to reduce the value of B, t should exceed 100 seconds in a bath regulated to $\pm$ 0·02°C. The value of B was negligible for the viscometers used in this work. In this case, therefore,

η = Kdt - solution viscosity *

η_0 = Kd_0t_0 - solvent viscosity.

η/η_0 = the solution/solvent viscosity ratio.

Now the most characteristic viscometric quantity when dealing with polymer solutions is the specific

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* The nomenclature used in this section is in accordance with that recommended by the International Union of Pure and Applied Chemistry, J. Polymer Sci., 8, 257, (1952).

viscosity (η_{sp}). This was defined by Staudinger as

$$\eta/\eta_0 - 1$$

Hence $\eta_{sp} = \frac{\eta - \eta_0}{\eta_0} = \frac{Kdt - Kd_0t_0}{Kd_0t_0}$

$$= \frac{t - t_0}{t_0}\text{, if } d = d_0$$ (an approximation which is valid for dilute solutions).

For polymer solutions η_{sp} is related to the concentration by the continued series

$$\eta_{sp} = Ac + Bc^2 + Cc^3 + \ldots\ldots$$

For low concentrations ($c < 1$ g./100 ml.) this reduces to

$$\eta_{sp} = Ac + Bc^2$$

which can be written as

$$\eta_{sp}/c = A + Bc.$$

where $\frac{sp}{c}$ is the viscosity number. It is the limiting case of this equation which is important, i.e.

$$\left[\frac{\eta_{sp}}{c}\right]_{\lim. c \to 0} = [\eta] = \text{the limiting viscosity number.}$$

This is obtained by extrapolation of the graph of $\frac{\eta_{sp}}{c}$ against c to zero concentration. The limiting viscosity number $[\eta]$ is characteristic of a solution, and is used to calculate the molecular weight of the solute.

Staudinger[43] was the first to propose that

$$[\eta] = KM$$

where K is a constant and M is the molecular weight of the solute.

This equation has now been superseded by one proposed by Mark[44] and Houwink.[45]

$$[\eta] = KM^a$$

an equation which appears to have wide application. K and a have to be evaluated, for every polymer/solvent system, from the viscosities of a series of well-defined fractions of known molecular weight. Only then can the equation be applied to the same polymer/solvent system with solutes of unknown molecular weight.

Viscosity gives rise to an average molecular weight defined as

$$\bar{M}_v = \left[\frac{\sum_i n_i m_i^{1-a}}{\sum_i n_i m_i} \right]^{\frac{1}{a}}$$

This average is identical with the weight average when $a = 1$. Since a is usually between 0·5 and 1·5, the viscosity average is nearer the weight average than the number average. So, when the constants K and a are being evaluated, the independent determination of the molecular weight should preferably be made by a method which gives a weight average.

Because of the difficulty of interpretation of results, viscosity is normally used only as a relative measure of the molecular weights of a series of samples. This has very useful applications in the control of preparative procedures in polysaccharide chemistry.

Apparatus and Procedure.

Viscosity determinations were made in a modified

FIG. 15

Modified Ubbelohde Viscometer

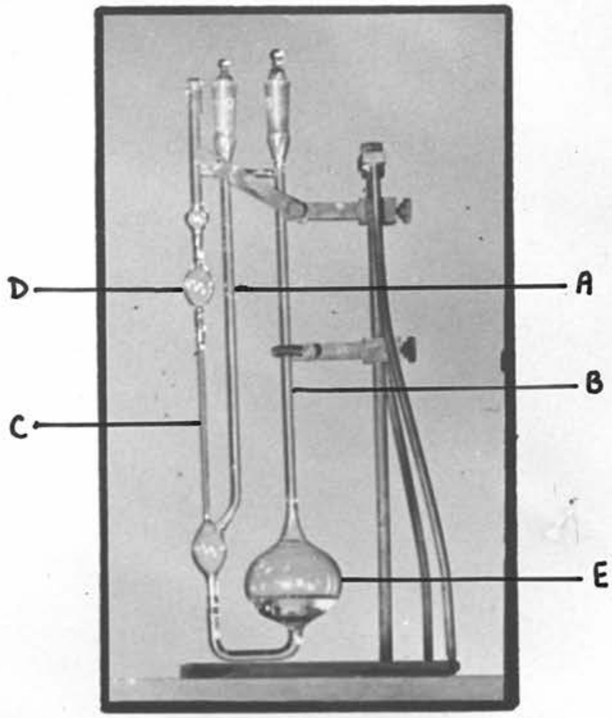
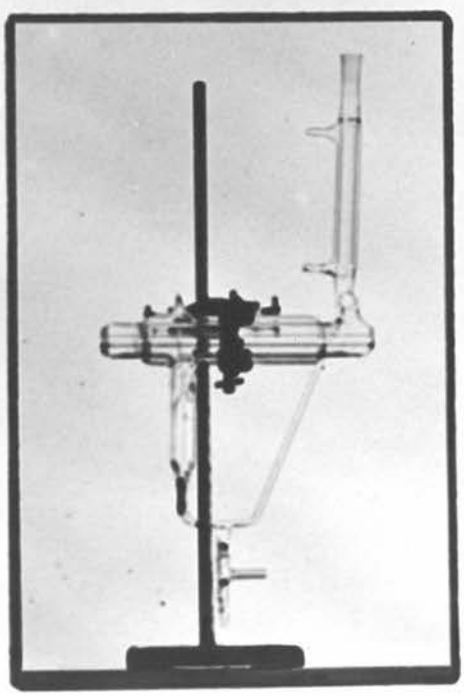


FIG. 16

Ebulliometer



Ubbelohde viscometer.[46,47] The apparatus (shown in Fig.15) was held vertical on a brass stand in the thermostat tank. 10 ml. of the original solution were added to the apparatus down tube (A). Ground glass sockets (B10) at the top of tubes (A) and (B) allowed tube (A) to be closed, with a ground glass stopper, while pressure was exerted at the top of (B). In this manner some of the contents of the viscometer were transferred to the bulbs at the top of the capillary (C) (0·4 mm. bore). When the pressure was released, the time for the liquid level to pass two marks, one above and one below bulb (D), was measured, using a stop watch reading to 0·05 seconds. The flow time of the solution was taken as the mean of at least three such replicate observations.

Dilutions were carried out in the viscometer. Accurately known volumes of solvent were added down tube (A), and the solution thoroughly mixed in bulb (E) by blowing gently down tube (A). At the end of the final measurement, the concentration of the solution was determined by removing a sample, and treating it as for solutions from an osmometer (see page 51).

The apparatus was thoroughly washed with solvent, and the flow time for solvent determined.

The solvent and the original solution were carefully filtered through G3 and G4 sintered glass filters before insertion into the viscometer. This was most important due to the ease with which the narrow capillary could be blocked by dust or debris from the solution or solvent.

EBULLIOMETRY.

Introduction and Theory.

The boiling point of a liquid is the temperature at which its vapour pressure is equal to the atmospheric pressure. Since the vapour pressure of a solution is lower than that of the solvent, at the same temperature (Raoult's Law $\frac{P_o-P}{P_o} = \frac{n}{n+n_o}$), the temperature at which the solution vapour pressure reaches atmospheric pressure is higher than the boiling point of the solvent.

The change in temperature (ΔT) needed to raise the vapour pressure of the solution to that of the solvent can be shown by thermodynamics to be

$$\frac{P_o-P}{P} = \frac{L\Delta T}{RT^2}$$

where P and P_o are the vapour pressures of solution and solvent respectively, L is the latent heat of vaporisation of the solvent, and T is the boiling point of the solvent.

Now $$\frac{P_o-P}{P} = \frac{n}{n_o}$$

where $n + n_o$ are the moles of solute and solvent, respectively, present in the solution.

$$\frac{n}{n_o} = \frac{L\Delta T}{RT^2}$$

From a knowledge of the concentration of the solution and the molecular weight of the solvent, the molecular weight of the solute can be determined.

The boiling point of a liquid, as measured by a thermometer immersed in the boiling liquid, has no real significance. Even if superheating of the liquid were eliminated, the thermometer would register a temperature higher than the boiling point. This is due to the hydrostatic pressure, at the level in the liquid at which the temperature is measured, causing an elevation of the boiling point above that at the surface.

The characteristic property of a boiling liquid is its boiling temperature. This is defined as the temperature of a thin film of liquid which is in equilibrium with its vapour, the vapour pressure being atmospheric pressure. Cottrell [48] was the first to realise this ideal with an apparatus which used the ebullition of the liquid to pump boiling liquid on to the thermometer. The liquid loses most of its superheating during the traverse of the pump, and the temperature registered on the thermometer is the boiling temperature.

The condensation temperature is the temperature of a thin film of condensed liquid, coexisting on the bulb of the thermometer with the vapour.

The condensation and boiling temperature of pure solvent are the same. If, however, non-volatile solute is added to the solvent, the boiling temperature only will be elevated. In this case, the difference between the boiling temperature and the condensation temperature is the elevation of the boiling point.

It is this temperature difference which is measured in the ebulliometer. The method has the advantage of being independent of changes in atmospheric pressure. There is one source of error, however, which is the increased concentration of the boiling solution, as compared with the solution before boiling commenced. The most convenient way to eliminate a correction for this is to make comparative measurements with substances of known molecular weight. This procedure was adopted.

Apparatus and Procedure.

Ray[49] has described an ebulliometer, which was claimed to be capable of measuring the elevation of the boiling point of solutions of polymers of molecular weights of up to 30,000. It was felt that this design had the disadvantage that the thermal ends, which were measuring the temperature elevation, were not sufficiently protected against the conduction of heat from one end to the other. In addition, a more ideal system would include a second apparatus in which solvent alone was boiling; the boiling temperature of the solution would then be compared with the condensation temperatures of the solution, and of the pure solvent in this second apparatus. Corrections could then be applied for changes in the condensation temperature of the solution due to volatile impurities in the solute.

With these ideas in mind, an apparatus was designed and constructed in which the condensation and boiling temperatures were measured in different wells. The boiling temperature well, which was heated by the out flow from a Cottrell pump, was made large enough to hold a second thermel. This could then be used to compare the boiling temperature of the solution with the condensation temperature of pure solvent in a second apparatus.

A twenty-junction thermel was made from 22 S.W.G. thermocouple wires (obtained from British Driver-Harris Co. Ltd., Manchester). The wires were varnished with a heat resistant insulating varnish. A galvonometer, sensitivity 105 mm./μA at one metre, and internal resistance 25Ω, although not sufficiently sensitive for measurements on polymers, was used in the preliminary experiments. Paraffin wax was used to ensure good contact between the thermel and the well. The whole apparatus was lagged with mineral wool.

When the apparatus was used with a 1% solution of α-naphthol as a standard solution, a temperature difference between the wells could be observed with a Beckmann thermometer. However, when the thermel and galvonometer were used no significant deflection was observed. The thermel sensitivity was determined, and found to be about 200 cm./$^{\circ}$C, which was sufficient for large deflections with the solutions used. It was observed that when the thermel had both ends at the same temperature, the galvonometer registered a considerable deflection. This effect could be explained on the basis of inhomogeneity of the wire.[50] An effect of this nature, however, should be superimposed on a deflection due to temperature differences between the thermel wells, and cannot account for the lack of response in the galvonometer.

In the apparatus, the thermel ends were in different wells, and the remainder of the thermel was outside the apparatus, at a much lower temperature, although the whole apparatus was lagged. This temperature differential was presumably such that heat was conducted away from the thermel so that the ends never reached the temperature of the wells.

When narrower gauge wires (36 S.W.G.) were used for the thermel, better results were obtained as there was less heat loss due to conduction, and perhaps the narrower gauge alloy was more homogeneous.

A second ebulliometer, taking account of all the above facts, was therefore designed as shown in Fig. 16

The apparatus was essentially three concentric glass tubes. The innermost tube, which held the thermel, was a 20 cm. length of specially selected thin walled Pyrex (0·8 cm. diameter). The middle tube, which was the vapour jacket round the thermel, was 2·5 cm. in diameter. The outer tube, 3·5 cm. in diameter, was a vacuum jacket insulating as much of the vapour jacket as possible. A side tube, 2 cm. in diameter, and 7·5 cm. long, held the Cottrell pump. The pump had a bell 1 cm. in diameter, and 4·5 cm. long. Boiling liquid was pumped up a 0·2 cm. tube, which was bent round so that the liquid was ejected on to the top of the thermel tube. The return from the condenser led to the heater, which was platinum wire wound, with a foundation of asbestos paper, on the glass below the side tube. The heater was supplied from a 10v. Variac transformer. A "T" piece, which could be sealed off with mercury, was attached to the foot of the condenser return. When the mercury level had been lowered the contents of the ebulliometer could be removed. Solute additions were made down the condenser.

A twenty junction thermel was constructed using 36 S.W.G. copper wire and 30 S.W.G. 'Eureka." The apparatus was then tested with the galvanometer described above. The galvanometer gave the expected deflections with standard solutions, and the apparatus appeared to behave quite satisfactorily in every way. However, experiments involving calibration and actual measurements on polymer solutions could not be started owing to the fact that the more sensitive galvanometer required was not available.

PART II

RESULTS OF THE DETERMINATIONS OF THE MOLECULAR WEIGHT OF POLYSACCHARIDES.

STUDIES ON INULIN.

INTRODUCTION

Fructosans occur in Nature as plant reserve carbohydrates, in particular they occur in the cell sap of the roots of the <u>Compositae</u>. Inulin is the fructosan which has been the subject of most investigations.

The most common sources of inulin are the roots of dahlias and chickory. Since inulin is in solution in the cell sap of these tubers, it is readily extracted in a relatively undegraded form. It can be dissolved out in warm water and then precipitated, either by cooling the solution, or by adding alcohol to the solution. Usually the plant juices are expressed, and collected before the macerated plant tissues are extracted. The enzymes in the combined extracts must then be inactivated to prevent inadvertent degradation of the inulin.

Enzymic hydrolysis of inulin has given a quantitative yield of D-fructose.[51] Methylation and hydrolysis have yielded principally 3:4:6-trimethyl fructose.[52] In view of these facts, and since the ease of hydrolysis indicates furanose rings, the interchain linkages in inulin have been accepted as being primarily of the 1-2 type.

The recently reported presence of chromatographically detectable quantities of glucose in the hydrolysates of various inulins;[52,53] the isolation of 2:3:4:6-tetra-O-methyl-D-glucose from methylated inulin;[52] and the biogenetic evidence that fructosans are built up in the plant from sucrose by transfructosidisation,[54] have all been indications that inulin, and other fructosans, contain a terminal glucose unit. Further evidence that the glucose is linked to the penultimate fructofuranose unit, as in sucrose, has come from the recent isolation of sucrose from solutions of autohydrolysed inulin.[55]

The molecular weight of inulin, as determined on inulin acetate by Rast's method was 6,300 (or 22 units).[56] Osmotic pressure measurements by Carter and Record[57] on the methylated and acetylated inulin were compared with the results of an end group assay. Both methods showed the chain length to be 30 units, and confirmed that inulin was a linear polysaccharide. The molecular weight of inulin, by osmotic pressure, has also been reported as 5,200 for an unpurified sample, and 7,000 for a purified sample.[58]

In the work reported here, inulin was extracted from dahlia tubers taking as many precautions as possible to avoid enzymic degradation and degradation due to the reagents. After expressing the juice, aqueous extracts of the tubers were carried out at various temperatures. No effort was made at this stage to remove protein although many such procedures have been reported.[52,54,59] It was thought that these might cause unnecessary degradation. Each fraction was examined chromatographically for glucose before being acetylated. The acetylated products were more stable than inulin which autohydrolyses in solution, and in addition, during the mild esterification protein was simultaneously removed. Acetylation was carried out using acetic anhydride after dispersing the inulin in pyridine. The acetylated products were examined by viscometry and isothermal distillation. Concurrently the effect of various conditions of acetylation were studied.

EXPERIMENTAL.

i) Isolation of Inulin Fractions.

Dahlia tubers (1 Kgm.) were peeled, washed, dried, and then minced. The minced product was collected in a muslin bag and the juice expressed (Fraction I). The residue after the expression of the juice was extracted for 5 minutes with cold water (1,500 ml. at 10-20 C), in an "Atomix" macerator. Solid matter was removed by centrifugation at 2,000 r.p.m. for 15 minutes, and the supernatant collected and filtered (Fraction II). The centrifugate was extracted with water (1,000 ml. at 50-60 C) in the "Atomix," and the extract separated from the solid matter, again by centrifugation and filtration (Fraction III). A final extraction with water at 90-100 C gave Fraction IV, and left a residue (weight = 10 g.). Each fraction was freeze dried.

All the fractions were analysed for percentage ash, nitrogen content (by semi micro Kjeldahl) and the viscosity (in aqueous solution). A sealed-tube hydrolysis with 0·5% HCl was carried out on each fraction, and the hydrolysed material qualitatively examined by chromatography. The reducing powers of the originals, and hydrolysed samples were determined by Somogyi's method.[60] The results are shown in Table 9.

Table 9

Fraction	I	II	III	IV
Total Weight (g.)	42·6	38·5	10	5
Ash (%)	4·5	5·9	1·6	3·6
Nitrogen (%)	0·89	1·4	0·47	0·40
Flow time (secs.) (1% solution in water)	253·9	226·5	240·3	237·5
Total Reducing Power (mg.Fructose/100 mg.Sample) (Unhydrolysed material)	2·8	3·8	1·6	1·1
Total Reducing Power (mg.Fructose/100 mg.Sample) (Hydrolysed material)	95	56	89	73
Chromatograms. (glucose/fructose)	1/50-100	1/30-40	Trace Glucose	No Glucose

ii) Acetylation of Inulin Fractions.

Inulin (2 g.) was swelled at 80°C for 1 hour in pyridine (20 ml.). Acetic anhydride (20 ml.) was added at the rate of 5-10 ml. per hour, at room temperature. The reaction mixture was stirred continuously until all the acetic anhydride had been added, after which it was transferred to a 100 ml. stoppered flask, and shaken overnight. After shaking, the solution was filtered through a grade 2 sintered glass filter and then poured into 100 ml. of iced water. The acetylated product was quickly filtered off, and thoroughly washed with cold water to remove the acetic acid.

The first stage in the purification of the acetate was to dissolve it in the minimum volume of acetone and reprecipitate it by adding water to the acetone solution. The acetate was centrifuged down, and further purified by dissolving it in the minimum volume of a 1:1 mixture of acetone and chloroform. Purified acetate was recovered by adding small volumes of this solution into petrol ether (b.p. 60-80°C). The acetate was dried overnight in a vacuum drier at room temperature.

iii) Examination of the Acetates.

The acetyl content of each acetate was determined by the method of Clark,[61] and all contained more than 40% acetyl. Since all the fractions were soluble in chloroform, the acetylation procedure was taken as satisfactory.

The molecular weight of the acetate of Fraction I was determined by isothermal distillation, and found to be 5,600 (see Table 10).

Table 10

Inulin acetate - Fraction I.

Solvent	Concentration (g./100ml.)	Time (hrs.)	(min.)	Change in solution level (mms.)	Change in solvent level (mms.)
Benzene	0·5	0	0	0·00	0·00
		18	10	0·76	0·79
		24	40	0·99	0·89
		43	10	1·44	1·44
		65	35	2·11	2·03
		90	55	3·06	2·89

$\bar{M}_n = 5,600$

The viscosity of chloroform solutions of each of the acetylated fractions was determined (see Table 11 and Fig.17).

Table 11

Inulin acetate - Fraction I.

Concentration (c g./100 ml.)	Specific Viscosity (η_{sp})	Viscosity Number ($\frac{\eta_{sp}}{c}$)
0·832	0·0463	0·0556
0·554	0·0312	0·0563
0·416	0·0239	0·0575
0·277	0·0166	0·0600
0.208	0·0125	0·0600
0		0·0630

Inulin acetate - Fraction II.

Concentration (c g./100 ml.)	Specific Viscosity (η_{sp})	Viscosity Number ($\frac{\eta_{sp}}{c}$)
0·952	0·0546	0·0574
0·634	0·0379	0·0598
0·381	0·0224	0·0588
0·317	0·0192	0·0606
0·238	0·0145	0·0609
0		0·0626

Inulin acetate - Fraction III

Concentration (c g./100 ml.)	Specific Viscosity (η_{sp})	Viscosity Number ($\frac{\eta_{sp}}{c}$)
1·044	0·0515	0·0493
0·696	0·0364	0·0523
0·522	0·0286	0·0548
0·348	0·0203	0·0583
0·261	0·0151	0·0579
0		0·0612

Inulin acetate - Fraction IV.

Concentration (c g./100 ml.)	Specific Viscosity (η_{sp})	Viscosity Number ($\frac{\eta_{sp}}{c}$)
0·968	0·0629	0·0650
0·646	0·0447	0·0691
0·484	0·0346	0·0714
0·323	0·0234	0·0724
0·242	0·0179	0·0741
0		0·0763

The values of $[\eta]$ were determined by extrapolation of the graph of $\frac{\eta_{sp}}{c}$ against c to zero concentration.

(iv) Effect of the Swelling Temperature on the Viscosity of Inulin Acetate.

Samples of inulin (Fraction I) were acetylated as above, except those in fraction IA and IB the inulin was swelled in pyridine at 50 C and 20 C respectively. The viscosity results for chloroform solutions are given in Table 12 and Fig.17.

Table 12

Inulin acetate - Fraction IA.

Concentration (c g./100 ml.)	Specific Viscosity (η_{sp})	Viscosity Number ($\frac{\eta_{sp}}{c}$)
0·892	0·0470	0·0527
0·594	0·0328	0·0551
0·446	0·0250	0·0560
0·297	0·0177	0·0595
0		0·0613

Inulin acetate - Fraction IB.

0·981	0·0369	0·0376
0·654	0·0257	0·0393
0·490	0·0198	0·0403
0·327	0·0148	0·0453
0		0·0477

The values of $[\eta]$ were determined graphically.

v) Further Acetylation Experiments.

Inulin acetate - Fraction IC was obtained by acetylating a sample of Fraction I as described, except that the acetate was recovered without shaking the reaction mixture overnight.

Inulin acetate - Fraction ID was prepared by dispensing inulin - Fraction I (2 g.) at room temperature in a mixture of formamide (20 ml.) and pyridine (20 ml.). The rest of the procedure was as for Fraction IC above.

The viscosity results for chloroform solutions are shown in Table 13 and Fig.17.

Table 13

Inulin acetate - Fraction IC.

Concentration (c g./100 ml.)	Specific Viscosity (η_{sp})	Viscosity Number ($\frac{\eta_{sp}}{c}$)
0·908	0·0421	0·0464
0·606	0·0304	0·0502
0·454	0·0242	0·0532
0·303	0·0161	0·0532
0·227	0·0127	0·0559
0		0·0595

Inulin acetate - Fraction ID.

Concentration (c g./100 ml.)	Specific Viscosity (η_{sp})	Viscosity Number ($\frac{\eta_{sp}}{c}$)
1·028	0·0530	0·0516
0·606	0·0366	0·0534
0·514	0·0228	0·0546
0·343	0·0208	0·0606
0·257	0·0156	0·0607
0		0·0635

The values of $[\eta]$ were determined graphically.

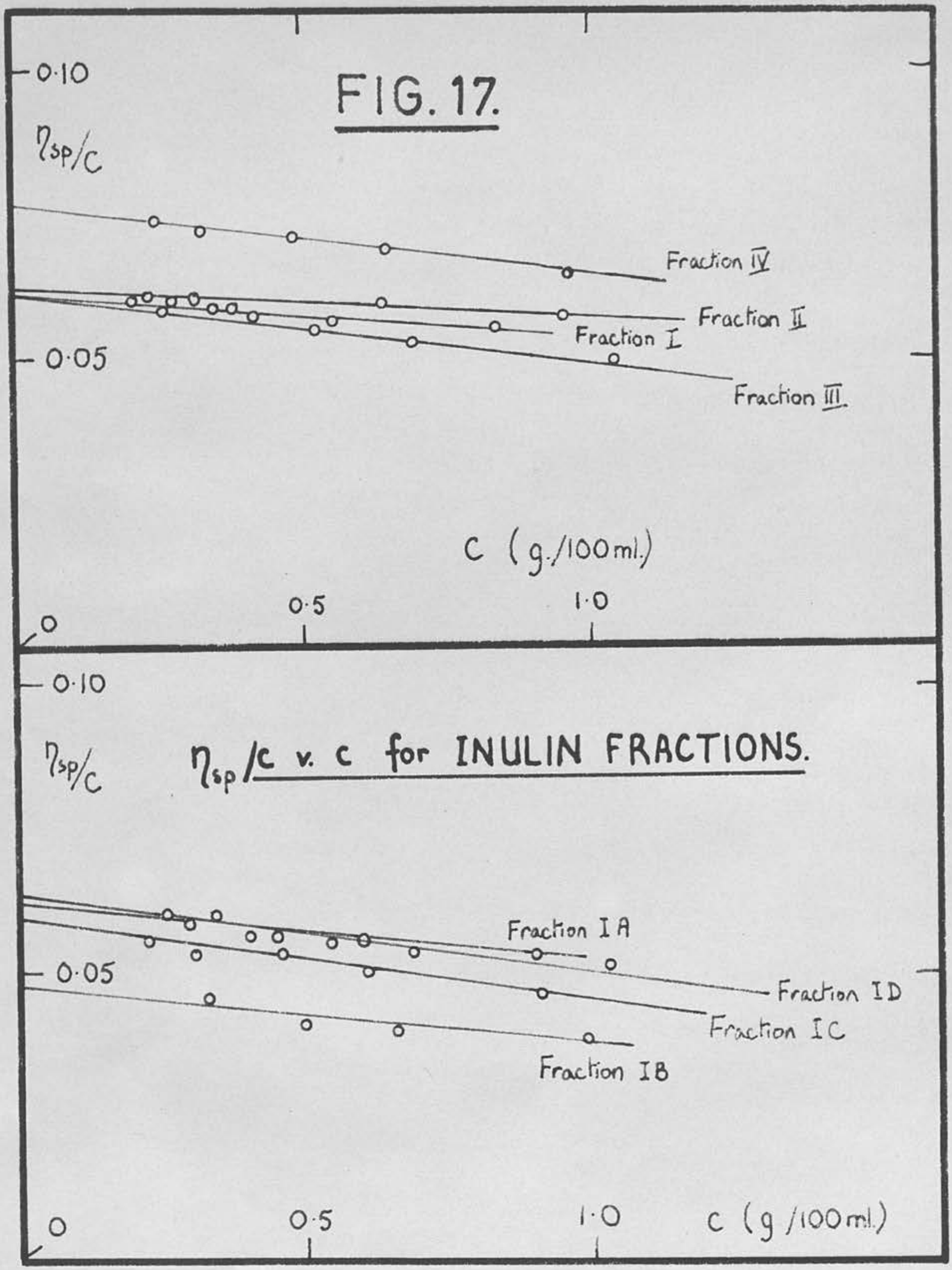


FIG. 17.

η_{sp}/c v. c for INULIN FRACTIONS.

DISCUSSION

A summary of the extraction scheme and the properties of the different fractions is shown in Table 14.

Table 14.

Results of Investigations on Inulin Fractions.

Fraction	% of tuber	Extraction Temp(C)	ηsp (H_2O)	M. Wt.	[η] of acetate	Amount of glucose
1	41	(Juice)	0·014	5,600	0·063	1/50-100
2	36	10-20	0·010	-	0·063	1/30-40
3	9	50-60	0·012	-	0·061	trace
4	5	90-100	0·011	-	0·076	none

The main bulk of inulin (77%) was extractable at room temperature. It is of interest that the amount of glucose present in the different fractions was small for the juice, increased for the cold water extract, and then decreased with increased extraction temperature. The complete absence of glucose at higher temperatures was presumably due to the autohydrolytic effects previously recorded.[55] The size of the fractions was only slightly dependent of the extractive temperature, which suggested that any degradation which might have occurred was of a stepwise hydrolytic nature.

Various modifications of the acetic anhydride/ pyridine method of acetylation were investigated (Table 15). It was found that swelling in pyridine at an elevated temperature appeared to cause degradation, whilst swelling at room temperature or dispersing in formamide were both successful. The latter procedure was then used to acetylate all the fractions (1-4). In view of

Table 15.

The Effect of Varying Acetylation Conditions.

Fraction	Swelling Temp.-reagent	$[\eta]$ of acetate
1A	50°C - pyridine	0·048
1B	20°C - pyridine	0·061
1C	15°C - pyridine	0·060
1D	15°C - formamide	0·064

their identical limiting viscosity numbers, the molecular weight of each fraction was not determined: that of fraction 1 was 5,600 (i.e. $\overline{D.P.}$ = 20). This value was slightly lower than those previously recorded (i.e. $\overline{D.P.}$ = 30[57] and 22[56]).

In view of the precautions taken to avoid degradation, the implications are either that the inulin molecule possesses a short chain length of about 20-30 units, or that its size may vary with the seasonal growth. In these investigations, the tubers were harvested in November in the dormant season. Further investigations could profitably be made on these lines.

STUDIES ON HEMICELLULOSES AND SHORT-CHAIN GLUCOSANS.

GENERAL INTRODUCTION

The polysaccharides to be considered in this section have been subdivided into four groups:

1. the hemicelluloses, or xylans,
2. the lichenins and isolichenins,
3. the laminarins,
4. other short-chain glucosans.

The hemicelluloses can be broadly defined as those polysaccharides, occurring along with cellulose in plant tissues. The most important group of these materials is the xylans - the only group of pentosans which has been studied to any great extent. Structural investigations have shown them to be usually short linear molecules.

Short-chain glucosans are also quite widely distributed in Nature. Few physical measurements of their size have been carried out. Structural investigations have usually shown them to be composed predominantly of unbranched chains of D-glucose units. The materials which were available for study included the laevorotatory glucosan from barley gum, laminarin, lichenin, and isolichenin.

1. THE HEMICELLULOSES

INTRODUCTION

Many polysaccharides occur in conjunction with cellulose in plant tissues. The term hemicellulose is usually applied to all the water insoluble carbohydrates of the cell wall, with the exception of cellulose and pectin. The bulk of hemicellulose material appears, however, to be a single polysaccharide-xylan, and this Department has been particularly interested in the isolation and examination of this polysaccharide.

The isolation of pure xylans has proved most difficult, due to the wide range of polysaccharides of differing molecular size and structure, which occur together as hemicellulose. In most cases the plant material is extracted with 5% alkali, sometimes at room temperature, but at other times higher temperatures are needed. Sometimes, but not always, a drastic delignification process (using sodium chlorite in acetic acid) is necessary before the alkali-soluble material can be isolated. Little is yet known of the effect of the reagents used in the extraction, on the molecular size of the product, but a recent review[62] has outlined the conditions necessary to avoid degradation during isolation.

After extraction, the product must be fractionated and purified. Processes such as acetone fractionation, ammonium sulphate fractionation, fractional solubilization and precipitation of the copper complex, are some which can be used. The success of any method depends to a large extent on the properties of the particular hemicellulose under consideration.

Structural investigations would appear to indicate that there are two types of xylans, 1) simple xylans, and 2) polymers of xylose incorporating glucuronic acid and arabinose. The structural significance of the glucuronic acid and arabinose have recently been questioned.[2]

The relation between the unit chain length, as derived by end group assay, and the physical molecular size has only seldom been reported,[63] but in one case[64] a structure involving one branch point was confirmed. In the main, structural investigations have indicated that the xylans possess a unit chain length of 200 or less. As a result the materials are difficult to deal with by osmometry, and the isothermal distillation method was almost invariably used.

EXPERIMENTAL.

The molecular weights of samples A1–A8 were determined by isothermal distillation (see Table 16).

Table 16.

Sample A1 - methylated xylan barley straw (solution in chloroform).

Concentration (g./100ml)	Time (hrs)	(mins)	Change in solution level (mm.)	Change in solvent level (mm.)
0·8	0	0	0·00	0·00
	3	0	0·07	0·06
	21	55	2·54	2·40
	28	35	3·45	3·19

$\bar{M}_n = 3,600$

Sample A2 - methylated xylan-oat straw (solution in benzene).

Concentration (g./100ml)	Time (hrs)	(mins)	Change in solution level (mm.)	Change in solvent level (mm.)
0·8	0	0	0·00	0·00
	17	51	-	0·31
	24	41	-	0·44
	41	54	-	0·79
	65	54	-	1·22
	89	54	-	1·62
	162	54	-	3·31

$\bar{M}_n$ = 14,700

Sample A3 - methylated xylan-esparto (solution in benzene).

0·8	0	0	0·00	0·00
	16	30	0·28	0·36
	74	0	1·96	2·15
	88	45	2·31	2·70
	114	20	3·00	3·61

$\bar{M}_n$ = 5,800

Sample A4 - methylated xylan-spruce (solution in benzene).

0·6	0	0	0·00	0·00
	14	40	0·24	0·34
	97	0	1·78	1·60
	121	0	1·90	2·48
	237	0	3·91	3·59

$\bar{M}_n$ = 16,900

Sample A5 - methylated xylan-barley husks (solution in benzene).

0·7	0	0	0·00	0·00
	24	0	0·55	0·38
	48	15	1·10	0·79
	72	15	1·75	1·29
	97	5	2·30	1·86
	128	55	2·92	2·67
	144	15	3·32	3·02

$\bar{M}_n$ = 10,500

Sample A6 - methylated xylan-wheat straw (solution in benzene).

Concentration (g./100ml.)	Time (hrs)	(mins)	Change in solution level (mm.)	Change in solvent level (mm.)
0·6	0	0	0·00	0·00
	23	50	0·52	0·42
	48	0	1·05	0·94
	72	5	1·47	1·45
	120	0	2·61	2·42
	144	0	3·03	3·06
	169	0	3·68	3·67
	192	0	4·22	4·25

$\bar{M}_n$ = 12,000

Sample A7 - methylated xylan-R.palmata (solution in benzene).

1·1	0	0	0·00	0·00
	17	0	0·88	0·34
	24	5	1·10	0·77
	40	45	1·83	1·50
	47	45	2·05	1·83
	65	0	2·98	2·59
	72	30	3·33	3·01
	89	20	3·97	3·73

$\bar{M}_n$ = 13,200

Sample A8 - acetylated xylan-R.palmata (solution in chloroform).

0·7	0	0	0·00	0·00
	17	50	0·36	0·28
	24	10	0·30	1·00
	74	35	1·86	2·42
	97	25	2·82	2·63
	113	45	2·66	3·06
	137	45	3·12	3·37
	161	45	3·54	3·76
	185	45	3·13	4·02

$\bar{M}_n$ = 62,100

The molecular weights of samples A9-A11 were determined by osmotic pressure measurements carried out with chloroform solutions of the polysaccharides. The

Gilbert, Graff-Baker, Greenwood osmometer was used for all three sets of measurements. No density corrections were applied to the observed osmotic pressures in view of the relatively large osmotic pressures which were developed. The results are given in Table 17 and Fig.18.

Table 17.

Sample A9 - acetylated xylan-holocellulose.

Concentration (c g./100 ml.)	Osmotic Pressure (π cm. solvent)	$\frac{\pi}{c}$
0·400	4·01	10·02
0·279	2·52	9·04
0·256	2·22	8·65
0·202	1·40	6·94
0·138	1·02	7·39
0	-	5·45

The equation of the graph of $\frac{\pi}{c}$ against c, as calculated by the method of least squares was:

$$\frac{\pi}{c} = 11{\cdot}65c + 5{\cdot}45, \text{ whence } \bar{M}_n = 31{,}000$$

Sample A10 - methylated xylan-oak heart wood.

Concentration (c g./100 ml.)	Osmotic Pressure (π cm. solvent)	$\frac{\pi}{c}$
0.804	11.48	14.28
0.595	7.46	12.54
0.505	6.24	12.36
0.431	4.93	11.44
0.352	4.09	11.62
0.304	3.18	10.46
0.193	1.97	10.17
0.146	1.46	10.00
0	-	8.94

The equation of the graph of $\frac{\pi}{c}$ against c, as calculated by the method of least squares was:

$$\frac{\pi}{c} = 6{\cdot}42c + 8{\cdot}94, \text{ whence } \bar{M}_n = 19{,}000.$$

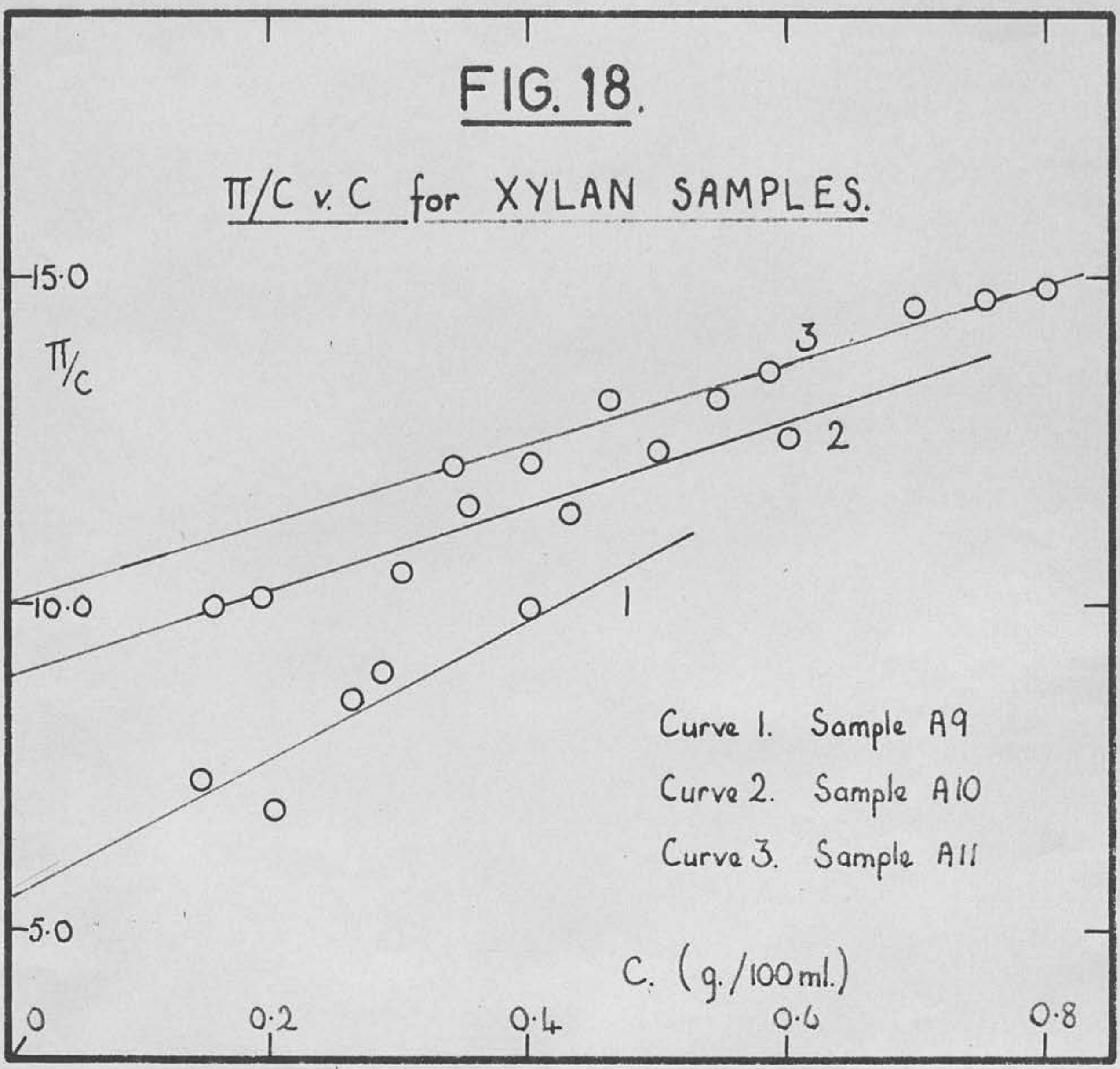


FIG. 18.
π/C v. C for XYLAN SAMPLES.
15·0
π/C
10·0
5·0
3
2
1
Curve 1. Sample A9
Curve 2. Sample A10
Curve 3. Sample A11
C. (g./100 ml.)
0
0·2
0·4
0·6
0·8

Sample A11 - reduced and methylated xylan-oak heart wood.

Concentration (c g./100 ml.)	Osmotic Pressure (π cm. solvent)	$\frac{\pi}{c}$
0·767	11·03	14·38
0·648	9·28	14·32
0·582	7·86	13·51
0·536	7·03	13·11
0·456	6·04	13·25
0·408	4·91	12·03
0·339	4·11	12·11
0	-	10·02

The equation of the graph of $\frac{\pi}{c}$ against c, as calculated by the method of least squares was:

$$\frac{\pi}{c} = 6{\cdot}05c + 10{\cdot}02, \text{ whence } \bar{M}_n = 16{,}800.$$

DISCUSSION

Table 18 summarizes the results of the molecular weight determinations. The results of chemical end-group assay (E.G.A.) are also shown in the instances where these are available, together with other comparable isothermal distillation results.

Table 18

Molecular Weight Determinations on Hemicelluloses.

Sample	Source	Derivative[a]	Solvent	Method[b]	$\bar{M}_n$	$\overline{D.P.}$	E.G.A.	REF.
A1	Barley straw	M	C_6H_6	I	3,600	23	-	96
A2	Oat straw	M	C_6H_6	I	14,700	92	45	97
A3	Esparto straw	M	C_6H_6	I	5,800	36	40	64
A6	Wheat straw	M	C_6H_6	I	12,000	75	50	98
-	Wheat straw	M	C_6H_6	I	8,000	50	40-45	99
A5	Barley husks	M	C_6H_6	I	10,500	66	-	100
A4	Spruce	M	$CHCl_3$	O.P.	16,900	106	100	101
A9	Beech holo-cellulose	A	$CHCl_3$	O.P.	31,000	195	200	102
-	Beech	M	C_6H_6	I	11,000	68	70	103
A10	Oak heartwood	M	$CHCl_3$	O.P.	19,000	119	100	104
A11	Oak heartwood	M	$CHCl_3$	O.P.	16,800	105	100	101
A7	*R.palmata*	M	C_6H_6	I	13,200	83	20	106
A8	*R.palmata*	A	$CHCl_3$	I	62,100	390	-	106

a, M = methylated; A = acetate;
b, I = isothermal distillation; O.P. = osmotic pressure.

The first group of xylans included several from different types of straw. The barley straw xylan (A1)[96] is unusual in that it possesses a relatively short unit chain-length. Although full details of chemical structure are not yet available it would appear to be a short linear xylan.

The oat straw xylan (A2)[97] has been isolated with cold aqueous sodium hydroxide ffom delignified tissue. Fractionation *via* the copper-complex yielded a product containing only a trace (3%) of arabinose and uronic acid. Methylation and hydrolysis showed that the length of the main chain was 45 xylose residues, whilst side-chains carrying the arabinose and uronic acid residues were also present. On purely chemical evidence, branching of the main xylose chain cannot be distinguished, but the molecular weight shows that the molecule must exist as a singly-branched structure.

A similar structure had been proposed for the esparto straw xylan (A3),[64] which had been isolated free from arabinose by several reprecipitations as the copper-complex. However, the molecular weight (i.e. $\overline{\text{D.P.}}$ = 36) in conjunction with the end-group assay of 40 shows the xylan is a linear molecule.

The wheat straw xylan (A6)[98] had been isolated by the direct extraction of the straw with alkali. The final product contained some arabinose and a trace of uronic acid residues. The end-group assay value of 50 compared with the $\overline{\text{D.P.}}$ of 75 suggests that whilst most of the xylan chains are linear, some at least must be branched. This is in contrast to another sample of wheat straw xylan,[99] which had been previously examined and shown to possess a short linear structure of 50 xylose residues.

The xylan from barley husks (A5)[100] had been isolated by direct extraction of the husks with alkali. Chemical evidence suggests that the molecule consists of a main xylan chain with side-chains carrying uronic and arabinose units, but detailed results are not yet available.

Straw xylans would appear in the main, therefore, to consist of relatively short linear chains of xylose residues with arabinose and uronic acid residues attached to this main chain. In some instances, branched molecules must also be present.

Several wood xylans were examined. The Norwegian spruce xylan (A4)[101] was unusual in that no polysaccharide was extracted from the sawdust unless delignification had been carried out. The delignified sawdust yielded, however, on extraction with cold alkali, a complex system of polysaccharides from which a xylan-rich fraction was obtained by copper-complexing. The xylan product also contained some uronic acid residues. Comparison of the methylation and hydrolysis results with the molecular weight suggests that a probable structure for the spruce xylan is a molecule of about 100 sugar residues with a main-chain of xylose units and single glucuronic acid residues attached to the chain by the extent of approximately 1 acid residue per 5 xylose units.

The beech holocellulose (A9)[102] sample had been shown to yield an exceptionally small amount of end-group on methylation and hydrolysis, and to possess, therefore, a very high molecular weight. However, the acetate in chloroform solution gave osmotic pressure results, which were consistent with a much smaller $\overline{\text{D.P.}}$ i.e. about 200. This structure is, however, still much larger than that of the previously examined hemicellulose A[103] of beechwood, a molecule which had been shown to consist of a straight chain of about 70 xylose residues.

The oak heartwood xylan (A10 and A11)[101,104] had been isolated by alkaline extraction followed by purification as the copper-complex. No prior delignification process was required. The xylan product contained 12% of uronic acid. Methylation and hydrolysis of sample (A10) gave a surprisingly low yield of end-group (0·2%) and a high yield of monomethyl xylose (4%). Since the latter might well have arisen from a branch-point or hydrolysis of the aldobiuronic acid, the material had been reduced with lithium borohydride in tetrahydrofuran (to reduce the -COOH groups, and so prevent monomethyl xylose arising, on hydrolysis), and this remethylated. The molecular weight of this reduced and remethylated product was found by osmotic pressure measurements to be 16,800 i.e. $\overline{\text{D.P.}}$ about 100. Since the original material had a $\overline{\text{D.P.}}$ of 115 (molecular weight = 19,000), there would appear to be little if any decrease in chain length during reduction and remethylation of the xylan. On the basis of these results, it appears that oakheartwood xylan consists of a main chain of about 100 xylose units to which glucuronic acid residues are attached; there being 1 acid residue per 10 xylose units.

The wood xylan samples would again appear to be essentially linear molecules, whose average length is greater than that of the straw xylans examined.

The algae xylan from Rhodymenia palmata (A7)[106] is unusual in that the chemical evidence suggests that both 1:3- and 1:4-linkages occur. The end-group assay value of 20 units compared with the $\overline{\text{D.P.}}$ of 83 suggests a branched structure. Although the $\overline{\text{D.P.}}$ value for the corresponding acetate (A8) i.e. 400, suggests that drastic degradation had occurred during the methylation, further work is necessary in view of the unsatisfactory nature of chloroform in isothermal distillations.

These results would indicate that the size of the xylan molecule is relatively small, although further work is necessary to investigate whether degradation occurs during isolation procedures or preparation of the methylated derivatives.

2. LICHENIN AND ISOLICHENIN

INTRODUCTION

The polyglucose lichenin is found to the extent of 10% in lichens, notably in Icelandic moss. The polysaccharide is easily extracted, being soluble in hot water. Structural studies involving methylation and hydrolysis,[65] and periodate oxidations[66,67] have shown it to be composed of D-glucose units linked, in the main, by β-1-4 bonds. However, about 30% of the bonds are β-1-3, which suggests an irregular chain in the molecule. This irregularity may be manifest in the differences between this polysaccharide and cellulose (also a β 1-4 glucosan) viz: the amorphous nature of lichenin and its ready solubility in hot water.

Few molecular weight determinations have been made on lichenins, but Carter and Record[57] have shown the osmotic molecular weight of a methylated derivative to be the same as the molecular weight determined by an end group assay. Thus they have proposed a linear structure for the molecule. These results have been confirmed by Meyer and Gürtler.[66]

Isolichenin is another polyglucose occurring in lichen. It is isolated simultaneously with lichenin, but since isolichenin is soluble in cold water the two polysaccharides are readily separable. Isolichenin gives a blue stain with iodine, which may be indicative of a structural relationship between this polysaccharide and starch. Structural investigations on this material are very limited and unreliable, because the extraction procedure probably gives a non-homogeneous polysaccharide.[68]

Aspinall, Hirst and Warburton,[69] have reported the preliminary examination of the alkali soluble polysaccharides from reindeer moss. The unfractionated polysaccharides have been subjected to methylation studies and to periodate oxidation. This work only emphasised the complexity of the unfractionated alkaline extract.

EXPERIMENTAL.

All the samples mentioned above have been subjected to molecular weight determinations by isothermal distillation in benzene solution (see Table 19).

Table 19

Sample B1 - methylated lichenin-icelandic moss.

Concentration (g./100ml)	Time (hrs)	(mins)	Change in solution level (mm.)	Change in solvent level (mm.)
1·4	0	0	0·00	0·00
	18	15	1·69	2·17
	19	35	1·78	1·97
	21	50	1·57	2·16
	39	35	3·27	3·50

$\bar{M}_n = 5{,}000$

Sample B2 - methylated isolichenin-icelandic moss.

0·9	0	0	0·00	0·00
	18	50	1·75	1·47
	24	50	2·29	1·76
	42	20	3·16	2·94
	48	5	3·45	3·35
	113	40	6·92	6·59
	161	40	9·12	8·86

$\bar{M}_n = 4{,}500$

Sample B3 - methylated alkali soluble polysaccharide-reindeer moss.

1·6	0	0	0·00	0·00
	6	55	0·01	0·10
	24	15	0·32	0·53
	31	45	0·51	0·60
	48	20	0·98	0·96
	54	50	1·18	1·08
	72	30	1·59	1·68
	96	25	2·21	2·17
	173	10	4·18	4·09

$\bar{M}_n = 22{,}000.$

DISCUSSION

The results of the observations on the polysaccharides from Iceland and Reindeer Mosses are shown in Table 20, together with the values from previous measurements.

It would appear that the Iceland Moss polysaccharides which had been examined in this Department had been extensively degraded - either during extraction or methylation. The results of other workers indicate that lichenin is a linear molecule. Since for both the lichenin and isolichenin samples, the $\overline{D.P.}$ was less than the chemical end-group assay value, it is not improbable that the samples studied were the whole methylated product, whilst a petroleum ether fractionation had been carried out before the E.G.A. had been attempted.

The D.P. value for the Reindeer Moss polysaccharide would suggest that this complex molecule consisted of approximately 100 sugar residues, and by comparison with the hypoiodite oxidation result, might be linear.

Further investigations on the molecular size of these materials are obviously necessary, especially with regard to the effect of the extraction and esterification procedures.

Table 20.

Results for Lichenin and Isolichenin.

Sample	Derivative[a]	Method[b]	Mol.Wt.	D.P.	E.G.A.	Ref.
Lichenin	M	I	5,000	24	70	106
"	A	O.P.	118,000	410	-	57
"	M	O.P.	10,700	52	80	57
"	F	C	26,000	160	160	66
Isolichenin	M	I	4,500	22	50	106
Reindeer Moss polysaccharides	M	I	22,000	100	(20,000)[c]	69

a: M = methylate; A = acetate; F = free polysaccharide.
b: I = isothermal distillation; O.P. = osmotic pressure; C = colorometric.
c, Results of hypoiodite oxidation.

3. THE LAMINARINS.

INTRODUCTION

Certain brown seaweeds contain a water-soluble polyglucose-laminarin. Laminarin can be isolated as an insoluble precipitate from the fronds of *L.claustoni* by leaching with acidified water.

Methylation studies show the polysaccharide to be a chain of glucose units, linked β-1-3,[70] and about 20 units long. The chain length has been confirmed by a molecular weight determination by Barger's method.[71]

L.digitata gives a laminarin with rather different properties, in that the polysaccharide is soluble in the acid leaching water, and must be precipitated with ethanol. Apart from this latter laminarin containing 3% fucoidin, there are no other structural differences between it and the laminarin from *L.claustoni*,[71] the chain length again being 20 units by end group assay and isothermal distillation.

Apart from the work of Percival and Ross,[71] the only other estimation of the molecular size of the polysaccharide was made by Cook with an ultracentrifuge.[72]

EXPERIMENTAL

The molecular weights of the various methylated derivatives of laminarin were determined by isothermal distillation in benzene (see Table 21).

Table 21

Sample C1 - methylated laminarin.

Concentration (g./100ml.)	Time (hrs)	(mins)	Change in solution level (mm.)	Change in solvent level (mm.)
1·2	0	0	0·00	0·00
	17	25	3·24	3·07
	19	25	3·58	3·52
	23	45	4·45	4·41

$\bar{M}_n$ = 1,900.

Sample C2 - methylated lime-treated laminarin.

0·3	0	0	0·00	0·00
	1	3	0·00	0·00
	16	15	0·59	0·65
	40	40	1·79	1·91
	64	15	2·91	3·30
	90	55	4·10	4·40

$\bar{M}_n$ = 2,500.

Sample C3 - methylated (petrol-ether precipitated) laminarin.

0·6	0	0	0·00	0·00
	16	5	0·17	0·56
	46	50	0·99	1·61
	65	5	1·53	2·11
	112	5	2·83	3·38
	137	35	3·46	4·14

$\bar{M}_n$ = 11,900.

Sample C4 - methylated (petrol-ether precipitated) lime-treated laminarin.

0·3	0	0	0·00	0·00
	17	30	0·22	0·09
	41	45	0·45	0·21
	65	30	0·66	0·41
	91	10	0·89	0·66
	114	45	1·02	0·93
	137	40	1·33	0·99
	163	5	1·56	1·21

$\bar{M}_n$ = 13,600.

Sample C5 - methylated laminaritol.

Concentration (g./100ml)	Time (hrs)	(mins)	Change in solution level (mm.)	Change in solvent level (mm.)
0·7	0	0	0·00	0·00
	15	0	0·99	0·87
	22	30	1·51	1·31
	37	30	2·64	2·37

$\bar{M}_n$ = 3,800.

Sample C6 - methylated (petrol-ether precipitated) laminarin.

0·4	0	0	0·00	0·00
	18	0	0·39	0·14
	41	30	0·58	0·41
	66	40	1·09	0·42
	89	15	1·18	0·91
	114	20	1·60	1·12
	163	45	2·09	1·75
	186	0	2·38	1·97

$\bar{M}_n$ = 12,100.

DISCUSSION

The results for the laminarin samples, which were all kindly provided by Dr. A. G. Ross, are shown in Table 22. The values were all obtained by isothermal distillation measurements on the methylated derivative.

Table 22.

Results for the Laminarin Samples.

Sample	$\bar{M}_n$	$\overline{D.P.}$	E.G.A.
1(a) Whole	1,900	9	-
(b) " , fractionation	(11,900 (12,100	58	20
2(a) Whole, $Ca(OH)_2$-treated	2,500	12	-
(b) " " " and fractionated	13,600	65	20
3 Laminaritol, fractionated	3,800	18	-

The sample of "insoluble" laminarin had been treated as follows:

1. it was methylated directly to give sample 1(a), and then fractionated with petroleum ether from chloroform solution to give sample 1(b) (78% yield).
2. a further portion had been treated under reflux with saturated lime-water at 60°C to yield a product (in 46% yield)which was unaffected by further lime treatments. This was methylated (sample 2a), and then fractionated with petroleum ether to give sample 2(b) in 88% yield.
3. a further portion had been reduced with lithium borohydride to yield laminaritol.

The results show that petroleum-ether fractionation results in the elimination of a considerable portion of low molecular weight material from the methylated material and that the fractionated products possess a $\overline{\text{D.P.}}$ much in excess of the end-group assay values. This is of considerable importance because it is the first clear evidence that laminarin is <u>not</u> a simple straight chain polysaccharide of about 20 glucose units in length.

It is of interest that similar results were obtained when aqueous solutions of both the unmethylated laminarin and lime-treated laminarin were examined by Dr. C. T. Greenwood in the Spinco electrically-driven ultracentrifuge. The Schlieren patterns obtained suggested that the laminarin had a very wide range of molecular weight - including higher molecular weight material - the sedimentation constant of the main bulk of material being

$0{\cdot}5 \times 10^{-13}$ c.g.s. units. Lime-treated laminarin on the other hand, was more homogeneous and possessed a larger sedimentation constant ($1{\cdot}0 \times 10^{-13}$ c.g.s. units).

Without further chemical evidence, it is difficult to draw any conclusion regarding the laminarin molecule, but it is certainly not simple.

4. OTHER SHORT-CHAIN GLUCOSANS.

INTRODUCTION.

Preece and MacKenzie[73] were the first to prepare a glucosan from barley grain, which was uncontaminated with pentosan. The structure of this polysaccharide has been described as unbranched chains of glucose units, linked in equal proportions β-1-3 and β-1-4.[74]

The α-glucosans which were examined were prepared by Aspinall and Fordyce[75] from esparto straw, but no structural data have yet been published. The results are shown in Table 23 below.

EXPERIMENTAL.

Sample D1 - methylated glucosan-barley gum.

Isothermal distillation in benzene solution.

Concentration (g./100ml.)	Time (hrs)	(mins)	Change in solution level (mm.)	Change in solvent level (mm.)
0·6	0	0	0·00	0·00
	17	45	0·14	0·32
	24	15	0·31	0·41
	41	45	0·63	0·81
	74	45	1·28	1·44
	97	10	1·57	1·83
	120	15	2·09	2·28
	161	45	3·04	3·06

$\bar{M}_n = 10,100$

Sample D2 - acetylated glucosan-esparto straw.

Isothermal distillation in chloroform solution.

0·7	0	0	0·00	0·00
	1	0	0·43	0·65
	2	35	1·29	1·44
	3	40	1·71	1·92
	4	40	2·10	2·21
	5	40	2·45	2·55

$\bar{M}_n = 2,100.$

Sample D3 - methylated glucosan-esparto straw.

Isothermal distillation in benzene solution.

Concentration (g./100ml.)	Time (hrs)	(mins)	Change in solution level (mm.)	Change in solvent level (mm.)
2·2	0	0	0·00	0·00
	1	0	0·88	0·86
	2	30	2·46	2·39
	3	30	3·48	3·31
	4	30	4·46	4·34
	5	30	5·68	5·10

$\bar{M}_n = 800$

Sample D4 - methylated glucosan-barley gum.

This sample was studied by osmotic pressure measurements, carried out with benzene solutions in the Zimm-Myerson osmometer. The membranes used were No.600 gel cellophane - non ammonia heated (see also page 71). No density correction was applied in view of the large pressures involved.

Concentration (c g./100 ml.)	Osmotic Pressure (π cm. solvent)	$\frac{\pi}{c}$
0·287	1·81	6·31
0·166	1·39	8·37
0·075	0·72	9·60
0·019	0·20	10·53
0		10·83

The equation of the graph of π/c against c, as calculated by the method of least squares, from the above data was $\frac{\pi}{c} = -15{\cdot}57c + 10{\cdot}83$, whence $\bar{M}n = 26{,}400$.

DISCUSSION.

Table 24 shows the results of the investigations. The barley glucosan was unusual in that the methylated product gave no tetramethyl glucose on hydrolysis. In view of the value of the $\overline{\text{D.P.}}$ (i.e. about 125 residues), and by comparison with the results for other water-soluble polysaccharides, a <u>linear</u> structure was suggested.

The glucosan from esparto grass had been extracted by aqueous leaching and fractionated <u>via</u> the copper complex. It had also been subjected to mild oxalic acid hydrolysis to remove arabinose, and might therefore have been degraded. The results indicate the material to be a short <u>linear</u> molecule.

Table 24.

Results for Other Glucosans.

Sample	Derivative[a]	Method[b]	M.Wt.	$\overline{\text{D.P.}}$	E.G.A.	Ref.
Barley gum (whole)	M	I	10,700	52	-	-
" " (fractionated)	M	O.P.	26,400	125	150	74
Esparto glucosan	M	I	800	4	4	75
" "	A	I	2,100	7	-	-

a, M = methylate; A = acetate:

b, I = isothermal distillation; O.P. = osmotic pressure.

STUDIES ON THE COMPONENTS OF STARCH.

INTRODUCTION

Starch is the most widely distributed food reserve carbohydrate in Nature. In the storage organs of plants the starch is laid down as characteristic granules, the size and shape of the granules being unique to the source.[76] Because of the importance of starch it has been extensively investigated by organic and biochemical methods, and as a result the main structural features, and the highly polymeric nature of the material are well established. Thus it is now well known that starch consists of long chains of D-glucopyranose units, linked in the main by α-1:4-bonds.

Many problems regarding the fine structure and the molecular size and shape of starch are still unsolved. It was in 1941 that Schoch[77] achieved the first quantitative separation of starch into two components, though the apparent inhomogeniety of starch had been indicated by the results of earlier workers.[78-82] Although starches can now usually be fractionated into amylose and amylopectin, the nature of the association of the molecular species in the starch granule is still by no means understood. As found in plant tissue the starch granules are surrounded by a thin layer of protein, but the nature of the synthesis and deposition of the starch in the granule, and the physical structure of the granule are still in the realms of speculation.

The two fractionated products of starch have, however, been studied, and it has been shown that while amylose is a mixture of essentially linear chains, amylopectin is a mixture of highly branched molecules.

The fractionation of starch has been achieved by, (1) electrophoretic separation of the charged amylopectin component in solution, (2) preferential precipitation of the amylose component from solution as a complex with a polar organic molecule, (3) preferential aqueous leaching of the amylose component from the granule.

The electrophoretic method does not give an efficient fractionation, and hence it has not been used to any great extent.[77]

The first quantitative separation of the components was made by forming the complex of amylose with n-butanol. This complex can be removed from the solution by high-speed centrifugation. Since the introduction of this method, many other reagents have been satisfactorily used to form insoluble complexes with amylose. The conditions necessary to achieve the maximum efficiency of fractionation, with the minimum inadvertent degradation of the labile amylose component have been discussed.[83] This method is generally thought to be the most satisfactory.

Although Schoch[77] has claimed that aqueous leaching of the amylose component is unsatisfactory recent work suggests that the method is of value in giving a high yield of undegraded amylose.[84]

In the work to be described in this section the problems which have been tackled are (1) the nature of the granular structure of potato starch, and (2) estimations of the size of the components obtained by different fractionation procedures from different starches. This work was carried out in collaboration with Mr. J. M. G. Cowie.

1. <u>THE NATURE OF THE GRANULAR STRUCTURE</u>.

In an effort to establish the nature of the outer "membrane" of starch granules, the action of dilute hydrochloric acid on the whole granules was examined. The effect of acid treatment on starch granules is not well established. Bauer and Pacsu[85] have utilized the degradative effect of hot acid on the granules to permit fractionation to be carried out at very high concentrations (10%). The resultant starch components were, however, highly degraded. Kerr[86] claims that acid treatment results in the preferential degradation of the amylopectin while amylose is relatively untouched. This might be explained on the basis of there being an outer "membrane" of amylopectin surrounding an inner core of amylose. On the other hand, Meyer and Menzi[87] claim that both components are degraded simultaneously, and that, consequently, amylose and amylopectin occur as "mixed crystals" in the granule.

The effect of the treatment of potato starch granules with 0·2N HCl at 40-45°C was followed by the subsequent fractionation of the components, and the determination of the molecular weights of their acetylated derivatives.

In a further series of experiments, potato starch granules were leached successively with water at 40, 70 and 90°C. The resultant products from this were precipitated from solution with butanol, purified by reprecipitation, and the size of the acetylated derivatives determined.

EXPERIMENTAL AND DETAILED RESULTS.

Preparation of Acid-Treated Starches.

A 2% suspension of potato starch (var. "Redskin") in 0·20N HCl was slowly stirred at 40-45°C in a nitrogen atmosphere. Portions were withdrawn after 1, 2, 3 and 4 hours respectively, each being washed free from acid by repeated sedimentation in distilled water, and finally shaken overnight in methanol.

The four samples were then fractionated by dispersion in boiling water (500 ml.) for 1½ hour with stirring under nitrogen. After cooling to 60°C, thymol (0·5 g.) was added. The mixture was allowed to stand for 3 days at room temperature, and then the resultant thymol-amylose complex was removed on the centrifuge. The supernatant amylopectin solutions were freeze-dried. The amylose-complexes were then purified by reprecipitating twice as the n-butanol complex.

The final amylose and amylopectin products were then characterized by measuring their iodine binding powers using the method described by Anderson and

Greenwood.[90] They were then acetylated with acetic anhydride in the presence of pyridine as a catalyst for amylose[83] and formamide for amylopectin.[83]

<u>Preparation of Aqueous-Leached Starches.</u>

A 0·5% suspension of potato starch (var. "Redskin") in water was heated with slow stirring under nitrogen for 1 hour at 40-45°C. After centrifugation, the granules were re-extracted at 50-55°C, and then at 70-72°C. Amylose was precipitated from the 70°C-extract by the addition of n-butanol and was then recrystallised to give amylose (A70). The residual material was fractionated using thymol as a precipitant (as above), following by three re-crystallizations with butanol to give amylose (A98). The two amyloses were then acetylated.

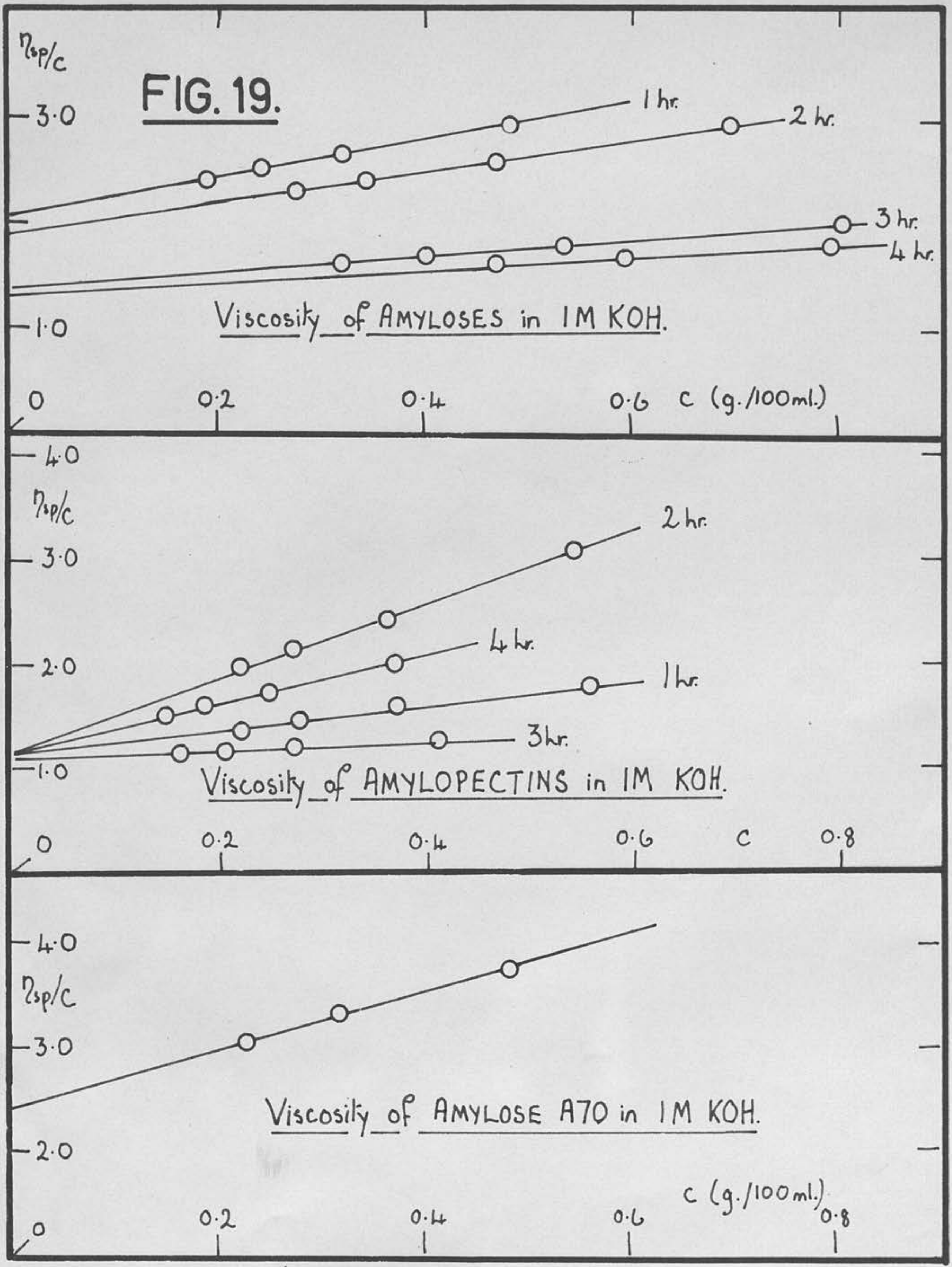
<u>Viscosity Measurements.</u>

The viscosity of the amyloses (samples 1-4) and amylopectins (samples 1-4) from the acid-treated starch were measured in 1M KOH solution, and the corresponding acetates in chloroform solution. These results are shown in Figs.19 and 20.

<u>Osmotic Pressure Measurements.</u>

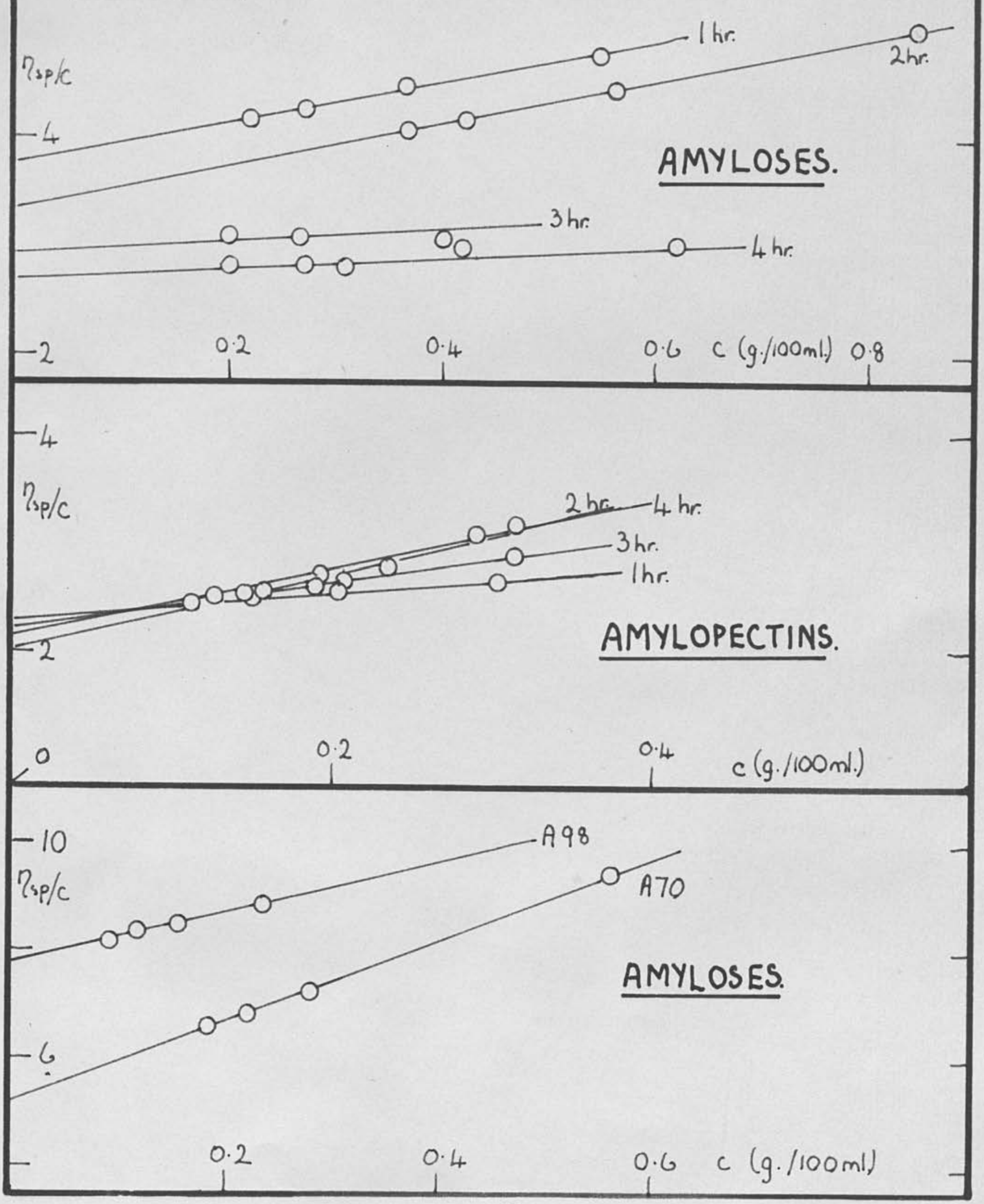
These were carried out on the acetylated derivatives in chloroform solution using the Gilbert-Graff Baker-Greenwood and the new osmometer. (See Table 25 and Figs. 21 and 22).

FIG. 19.



η_{sp}/c
3.0
1 hr.
2 hr.
3 hr.
4 hr.
1.0
0
0.2
0.4
0.6
c (g./100ml.)
Viscosity of AMYLOSES in 1M KOH.
4.0
η_{sp}/c
3.0
2 hr.
4 hr.
2.0
1 hr.
3 hr.
1.0
Viscosity of AMYLOPECTINS in 1M KOH.
0
0.2
0.4
0.6
c
0.8
4.0
η_{sp}/c
3.0
Viscosity of AMYLOSE A70 in 1M KOH.
2.0
c (g./100ml.)
0
0.2
0.4
0.6
0.8

FIG. 20. VISCOSITIES of ACETATES in CHLOROFORM.



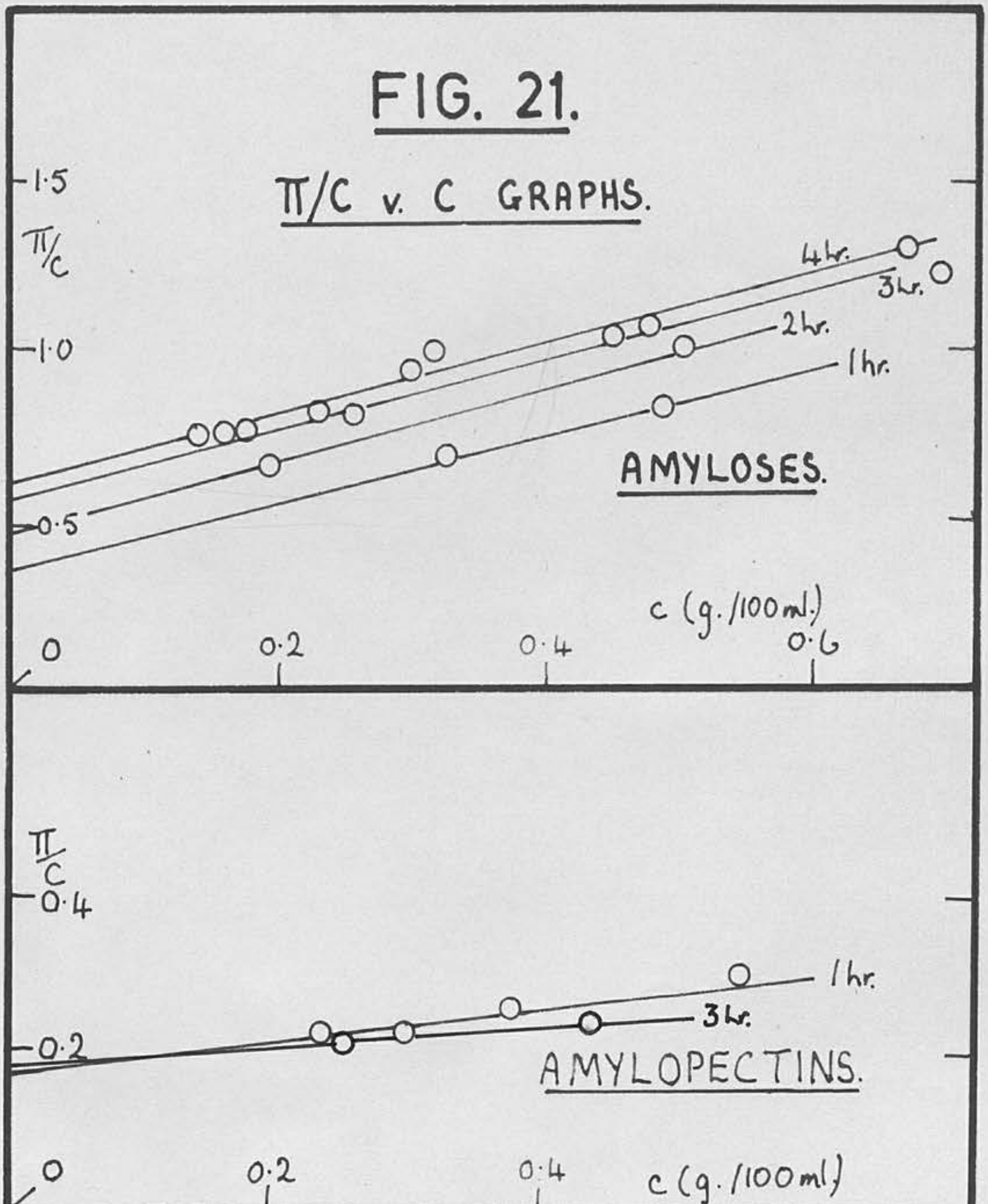


FIG. 21.
π/C v. C GRAPHS.
π/c
1·5
1·0
0·5
4hr.
3hr.
2hr.
1hr.
AMYLOSES.
c (g./100ml.)
0
0·2
0·4
0·6
π/c
0·4
0·2
1hr.
3hr.
AMYLOPECTINS.
0
0·2
0·4
c (g./100ml.)

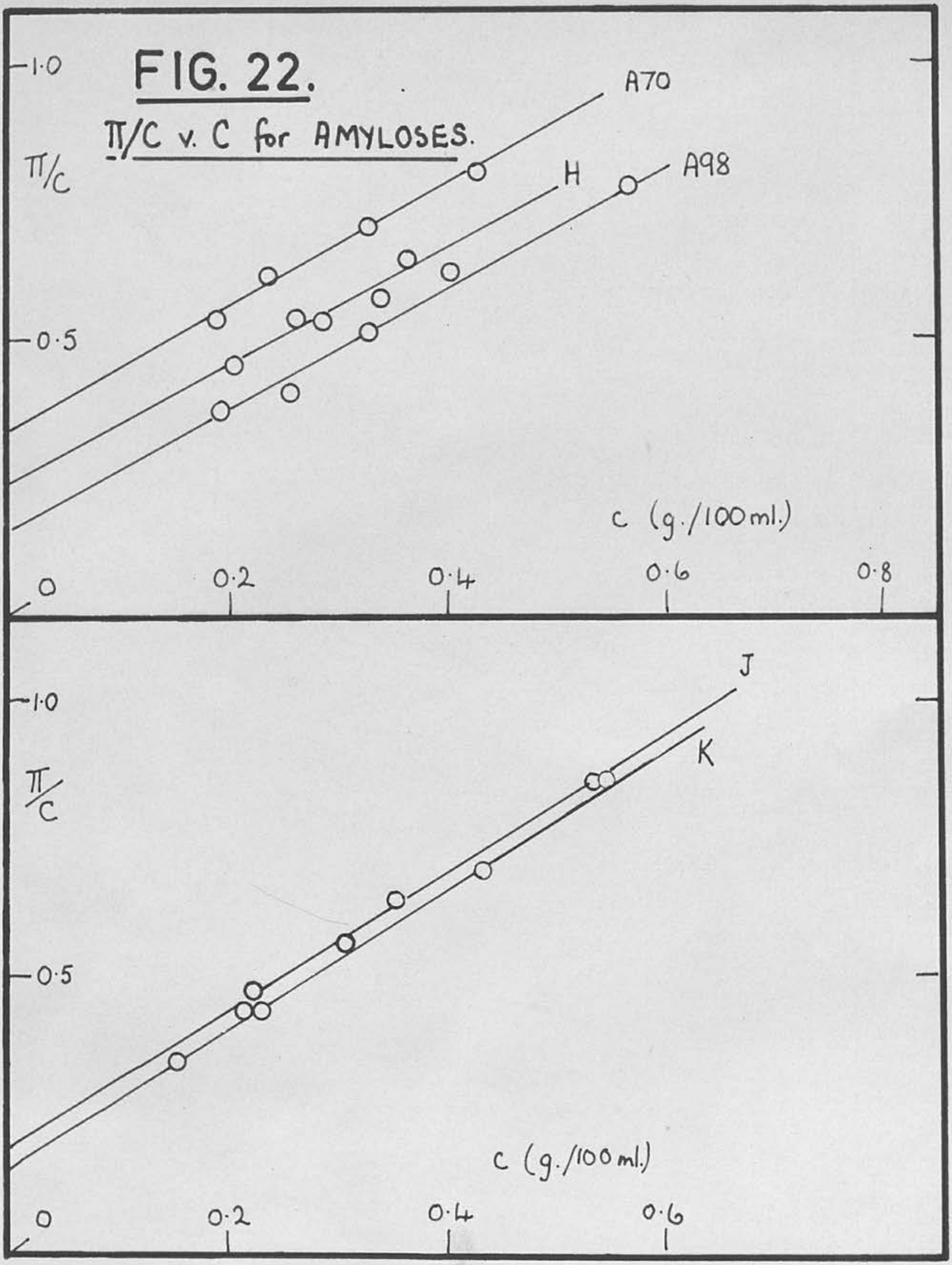


FIG. 22.
Π/C v. C for AMYLOSES.
Π/C
1·0
0·5
A70
H
A98
c (g./100ml.)
0
0·2
0·4
0·6
0·8
1·0
Π/C
0·5
J
K
c (g./100ml.)
0
0·2
0·4
0·6

Table 25.

Acetylated Amylose 1

c (g/100ml.)	π (cms.)	π/c
0·485	0·403	0·83
0·326	0·223	0·68
0	-	0·36

$\bar{M}_n$ = 470,000

Acetylated Amylose 2

c (g/100ml.)	π (cms.)	π/c
0·502	0·497	0·99
0·300	0·284	0·95
0·196	0·128	0·65
0	-	0·42

$\bar{M}_n$ = 400,000

Acetylated Amylose 3

c	π	π/c
0·679	0·873	1·29
0·476	0·511	1·07
0·358	0·407	1·14
0·175	0·134	0·77
0	-	0·56

$\bar{M}_n$ = 302,000

Acetylated Amylose 4

c	π	π/c
0·453	0·471	1·04
0·315	0·313	0·99
0·255	0·202	0·79
0·160	0·121	0·76
0·132	0·097	0·73
0		0·60

$\bar{M}_n$ = 286,000

Amylopectin Acetate 1

c	π	π/c
0·549	0·169	0·31
0·380	0·094	0·25
0·297	0·060	0·20
0·235	0·050	0·21
0	-	0·15

$\bar{M}_n = 1·1 \times 10^6$

Amylopectin Acetate 3

c	π	π/c
0·489	0·188	0·24
0·231	0·048	0·21
0	-	0·17

$\bar{M}_n = 1·0 \times 10^6$

Amylose A70 Acetate

c	π	π/c
0·427	0·342	0·80
0·327	0·230	0·70
0·236	0·145	0·61
0·190	0·102	0·54
0	-	0·34

$\bar{M}_n$ = 495,000

Amylose A98 Acetate

c	π	π/c
0·401	0·251	0·63
0·326	0·168	0·52
0·258	0·103	0·40
0·190	0·072	0·38
0	-	0·15

$\bar{M}_n$ = 1,500,000

DISCUSSION

The effect of the acid treatment on the iodine binding power of the starch samples is shown in Fig.23; a decrease in the apparent percentage of amylose and an

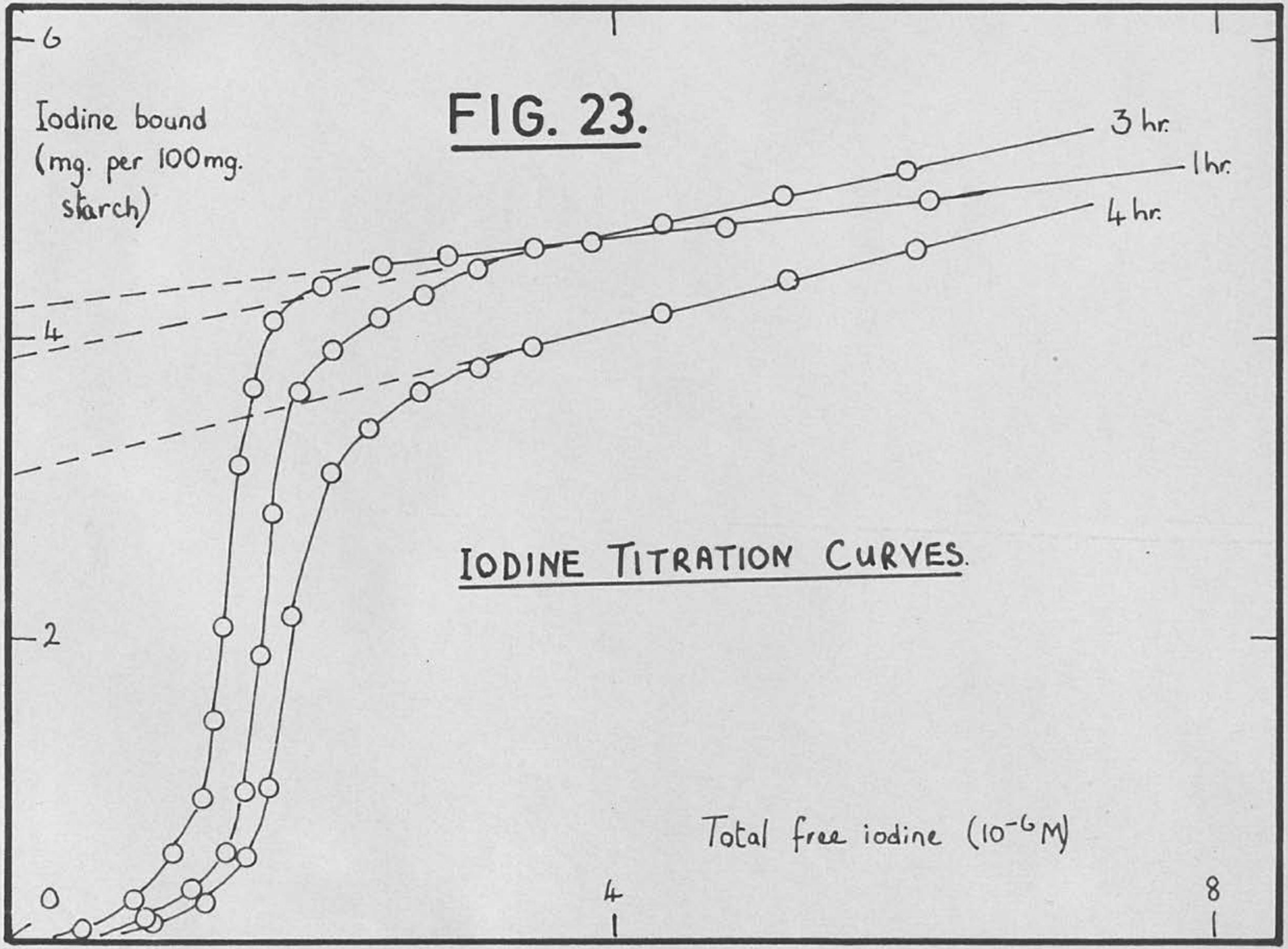


FIG. 23.

IODINE TITRATION CURVES.

increase in slope of the linear portion of the iodine binding curve occurs. The exact significance of the slope of the linear portion of this curve is not known with certainty,[90] but it may indicate the presence of short linear amylose chains. This result differs from that of Meyer and Menzi.[87] These authors found that a similar acid treatment did not affect the iodine binding power; but this can only indicate that their apparatus was insufficiently sensitive to detect such differences as have been found here. (A similar affect has been found for other starches examined by Mr. A. Arbuckle).

Table 26 summarises the data obtained from measurements on the fractionation products from the acid treated starches. It is apparent that the amylose component has been preferentially degraded whilst the amylopectin hâs remained virtually unchanged.

Table 26

The Properties of the Fractionated Components of the Acid Treated Starches

Component	Time of acid treatment (hr.)	$[\eta]$ 1M KOH	$[\eta]$ Acetate in $CHCl_3$	$\bar{M}_n$*	$\overline{D.P.}$
Amylose	0	5.00	6·80	1,111,000	3,870
Amylose	1	2·10	3·75	470,000	1,630
"	2	1·90	3·35	400,000	1,390
"	3	1·40	2·90	302,000	1,050
"	4	1·30	2·70	286,000	1,000
Amylopectin	1	1·10	2·20	1,100,000	3,800
"	2	1·15	2·00	-	-
"	3	1·08	2·20	1,000,000	3,500
"	4	1·15	2·05	-	-

(The size of the amylose component refers to that material which can be precipitated with butanol; any degraded

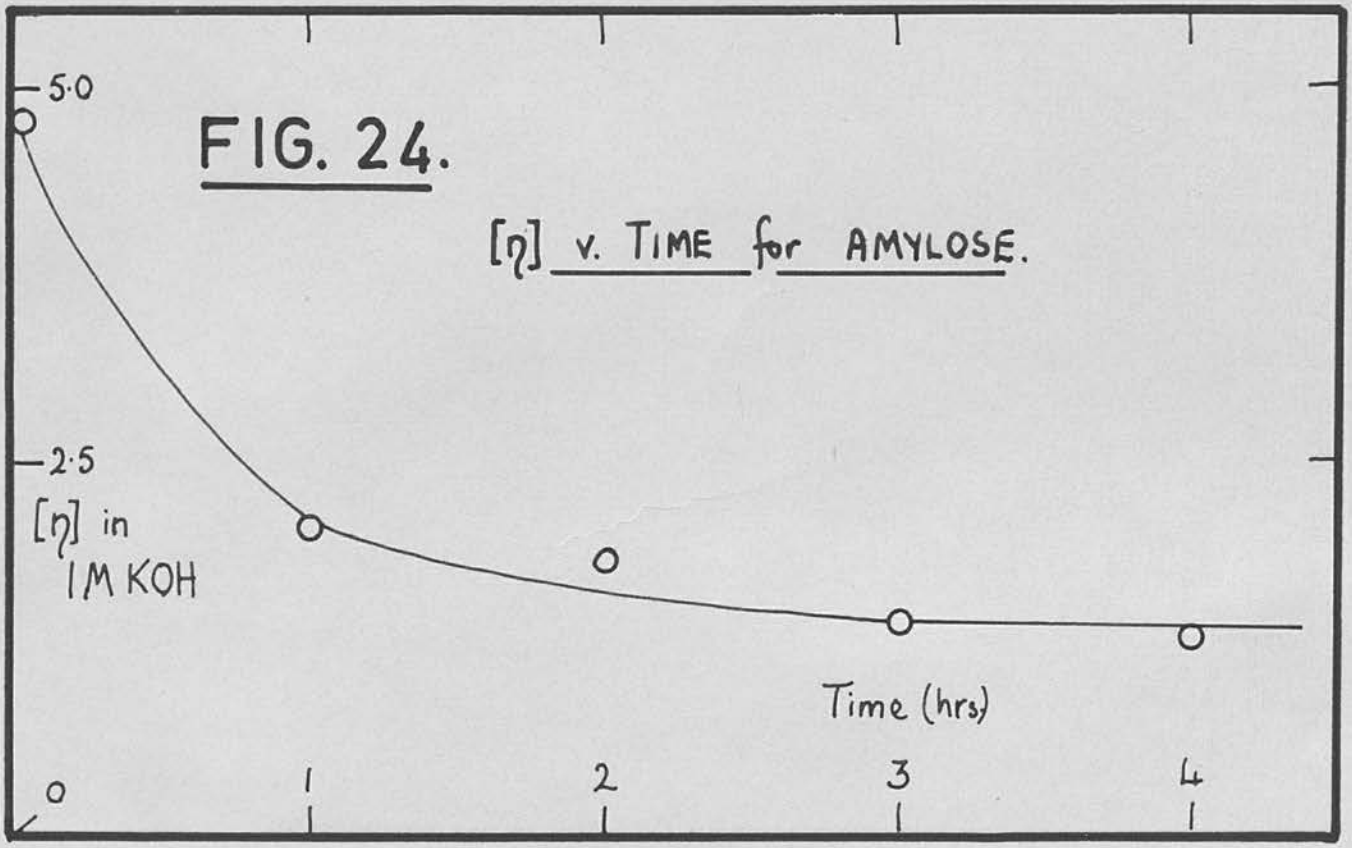


FIG. 24.
[η] v. TIME for AMYLOSE.
5·0
2·5
[η] in
1M KOH
Time (hrs)
0
1
2
3
4

linear material would be lost during the working up procedure). As shown in Fig.24, the decrease in molecular size of the amylose approaches a limiting value after about four hours' treatment, but the number of bonds broken per initial amylose molecule (i.e. about three) is not very great.

It is apparent therefore that the acid treatment has not caused either preferential degradation of the amylopectin (see ref. 86), or simultaneous degradation of both components (see ref. 87); rather would it appear to cause preferential hydrolysis of the amylose. This is supported by the results of the potentiometric iodine titration curves on the whole starches.

The irregular variation in slope of the amylopectin viscosity curves for the free component was reproducible but no explanation can be advanced for this.

In view of the fact that Kerr[86] characterised his materials in terms of the "fluidity" of the degraded starch samples, it is difficult to compare the properties of his products with those obtained here. However, the reported preferential degradation of the amylopectin and stability of amylose to acid-treatment was not found.

These results also differ completely from those of Meyer and Menzi.[87] The molecular weights quoted by these authors were entirely dependent on measurements of reducing powers, and it has been conclusively shown[91] that such methods are unsatisfactory for high molecular weight materials, and hence the absolute values given by Meyer and Menzi are not likely to be correct.

The attack of acid on the granule is not uniform; a result which is not surprising in view of the high degree of organisation of the granule. The results do not support Meyer and Menzi's idea of "mixed crystals of amylose and amylopectin," nor do they indicate a preferential attack on the amylopectin as might be expected if the outer layers of the granule were composed of this component. The action of acid must be complex.

The results of determinations of the molecular size of the aqueous leached products is shown below.

Sample	Temp. of Extraction (°C)	$[\eta]$ in 1M KOH	$[\eta]$ of acetate in $CHCl_3$	$\bar{M}_n$	$\overline{D.P.}$
A70	70	2·40	5·10	495,000	1,700
A98	98	-	7·80	1,500,000	5,200

It would appear that as the extraction temperature is increased so the $\overline{D.P.}$ of the amylose product increases. At 40°C, the amount extracted was too small for examination, at 70°C relatively short chain amylose is leached, whilst the remaining amylose obtained by a conventional 98°C dispersion has a higher than usual $\overline{D.P.}$ The granular structure would therefore appear to be such that on swelling in water relatively small amylose molecules are able to diffuse out from the internal structure; a fact which is substantiated by microscopic examination. If amylose is so readily leached from the granule, perhaps it is not surprising that on treatment with acid this component is preferentially attacked.

Further work on these problems is obviously necessary before the mode of attack of acid on the intact granule can be unambiguously described, and before more evidence for granular structure can be obtained.

2. THE SIZE OF THE FRACTIONATED STARCH COMPONENTS.

INTRODUCTION

In view of the recent investigation of aqueous leaching as a method of fractionation of starch,[84] and because of Meyer's[88,89] claims that leaching has separated potato and maize amyloses into two fractions of greatly differing $\overline{D.P.}$ it was of interest to compare the products of fractionation by leaching at 95°C for 10 minutes, and of fractionation by the conventional thymol and butanol complex formation. Also considered was the effect of Meyer's and other fractionation procedures on potato starch.

Molecular weight data were found for the acetylated components from potato and rubber seed starches, in order to compare the values with others reported in the literature.

Detailed Results and Experimental.

Fractionation Experiments on Potato Starch.

a) Aqueous Leaching (as described by Baum and Gilbert[84]).

Potato starch (*var.* Redskin, 10 g.) was treated for 7.5 mins. with 0·1% NaCl solution (2·5 l.) at 98°C in a nitrogen atmosphere. The swollen granules were then removed on the Sharples supercentrifuge, washed three times with distilled water and re-extracted for 7·5 mins. at 98°C. The amylose in the supernatant liquor was precipitated in the presence of butanol. Purification was achieved by reprecipitating three more times to yield amylose (H). The product was acetylated.

b) Successive Aqueous Leaching (as described by Meyer).

This has been described in the previous section (p.127).

c) Thymol-and Butanol-Fractionation.

Potato starch (var. Redskin, 15 g.) was dispersed with vigorous stirring under nitrogen in 0·1% NaCl solution (3 l.) at 98°C. Heating was continued for 2 hrs., after which the solution was allowed to cool to 60°C and thymol (5 g.) added. After standing at room temperature for 3 days, the thymol-amylose complex was removed on the centrifuge and purified by three reprecipitations as the butanol complex to give amylose (J).

Amylose (K) was prepared similarly except that the time of dispersion at 98°C was altered to 1 hr.

Both amyloses were then acetylated.

Viscosity Measurements.

Results of viscosity determinations of the free amyloses in 1M KOH and the acetylated derivatives in $CHCl_3$ solution are shown in Fig.25.

Osmotic Pressure Measurements.

Results are shown in Table 27 and Fig.22.

Table 27

Amylose H			Amylose J			Amylose K		
c	π	π/c	c	π	π/c	c	π	π/c
0·365	0·234	0·64	0·537	0·453	0·84	0·548	0·470	0·86
0·343	0·196	0·57	0·353	0·225	0·64	0·432	0·300	0·69
0·288	0·153	0·53	0·222	0·106	0·47	0·309	0·173	0·56
0·265	0·141	0·53	0·218	0·095	0·44	0·234	0·102	0·44
0·209	0·094	0·45	0	-	0·19	0·151	0·052	0·34
0	-	0·25				0	-	0·15
$\bar{M}_n$ = 670,000			$\bar{M}_n$ = 888,000			$\bar{M}_n$ = 1,110,000		

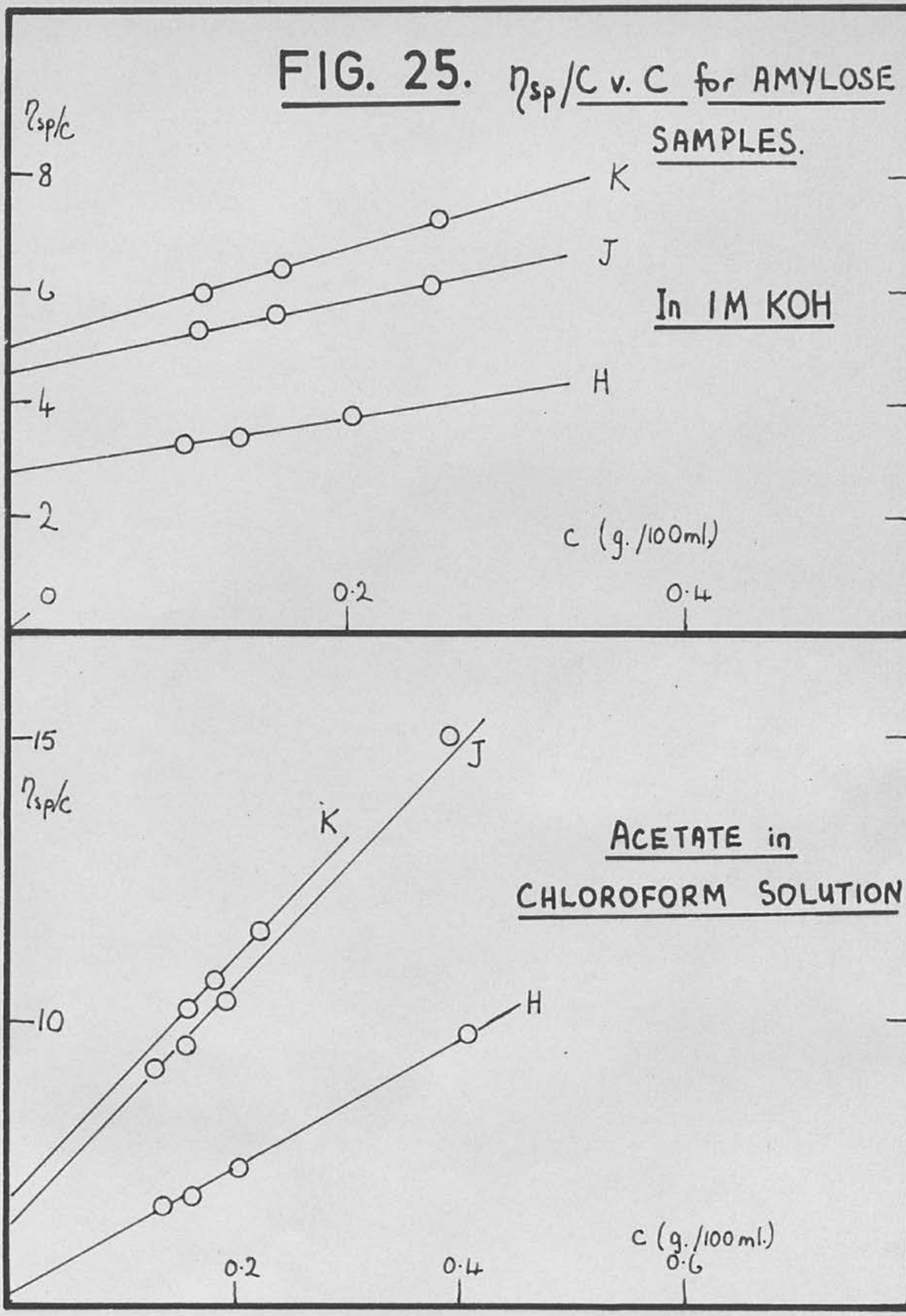


FIG. 25. η_{sp}/C v. C for AMYLOSE SAMPLES.
η_{sp}/c
8
6
4
2
0
K
J
H
In 1M KOH
c (g./100ml.)
0·2
0·4
15
10
η_{sp}/c
J
K
H
ACETATE in CHLOROFORM SOLUTION
c (g./100ml.)
0·2
0·4
0·6

Measurements on Another Sample of Potato Amylose.

This sample had been provided by the courtesy of Dr. G. A. Gilbert and had been fractionated using butanol as a precipitant under a nitrogen atmosphere. The results of osmotic and viscosity determinations are shown in Table 28.

Table 28

c	π	π/c	c	η_{sp}	$\frac{\eta_{sp}}{c}$
0·642	0·538	0·836	0·436	4·514	10·35
0·573	0·441	0·770	0·385	3·957	10·28
0·441	0·286	0·649	0·291	2·574	8·85
0·407	0·241	0·592	0·259	2·144	8·28
0·301	0·158	0·526	0·218	1·750	8·03
0·266	0·123	0·459	0·193	1·455	7·54
0·215	0·090	0·418	0·146	0·989	6·77
0·189	0·063	0·334	0·129	0·868	6·73
0·119	0·033	0·280	0·109	0·694	6·37
0·060	0·016	0·264	0·096	0·603	6·28
0	-	0·155	0	-	5·06

$\bar{M}_n = 1,100,000$

Measurements on Amylose and Amylopectin from rubber seed starch.

These results are shown in Table 29 and Fig.27.

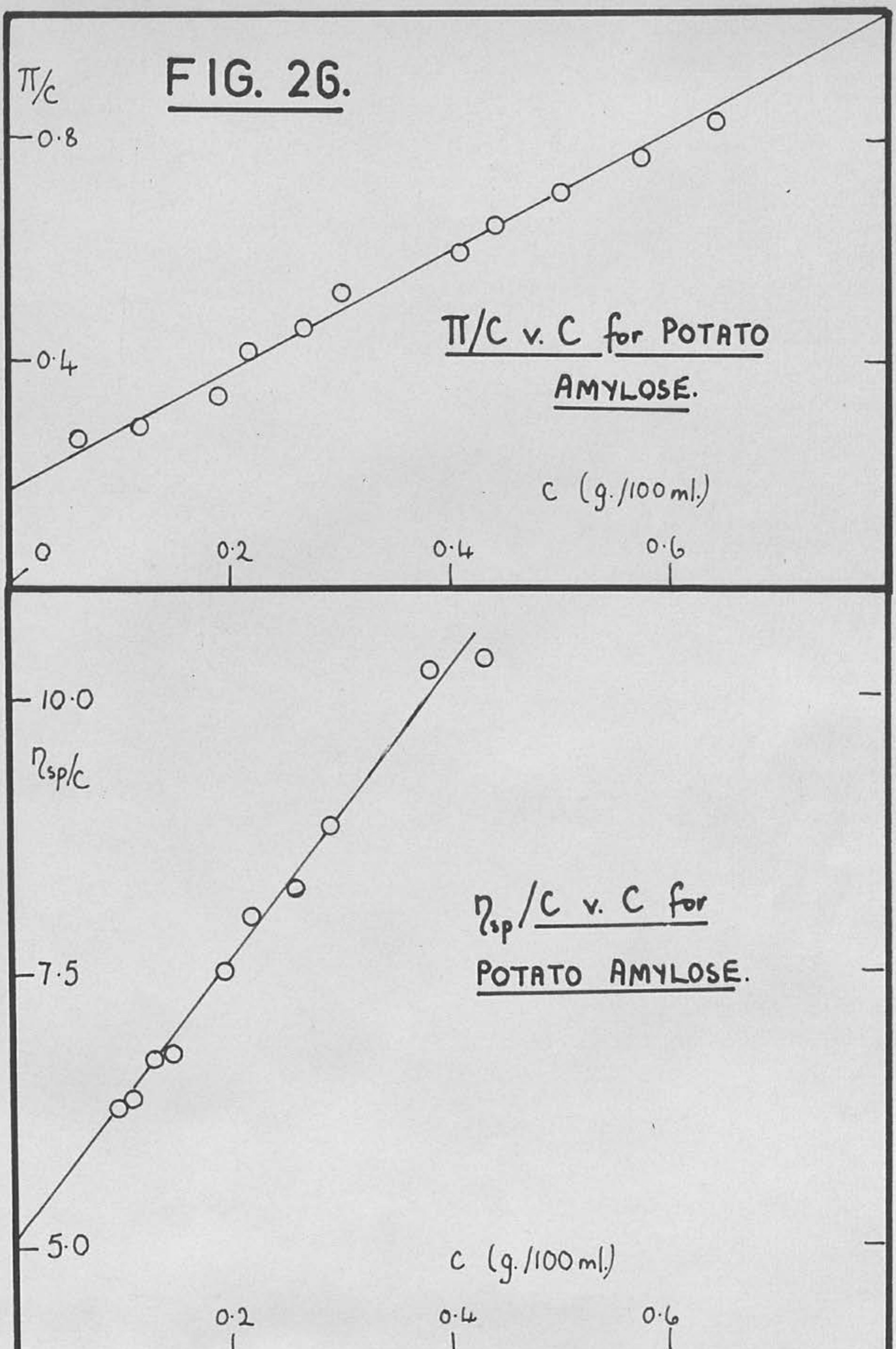
Table 29

Acetylated amylopectin.

Concentration (c g./100 ml.)	Corrected Osmotic Pressure (π cms. solvent)	$\frac{\pi}{c}$
0·6735	0·071	0·106
0·5099	0·052	0,102
0·4349	0·045	0·104
0·2305	0·020	0·098
0·1598	0·016	0·100
0	-	0·097

The limiting value of $\frac{\pi}{c}$ as $c \rightarrow o$ was found by extrapolation to zero concentration of the graph of $\frac{\pi}{c}$

FIG. 26.



π/C
0·8
0·4
π/C v. C for POTATO AMYLOSE.
c (g./100 ml.)
0
0·2
0·4
0·6
10·0
ηsp/C
7·5
5·0
ηsp/C v. C for POTATO AMYLOSE.
c (g./100 ml.)
0·2
0·4
0·6

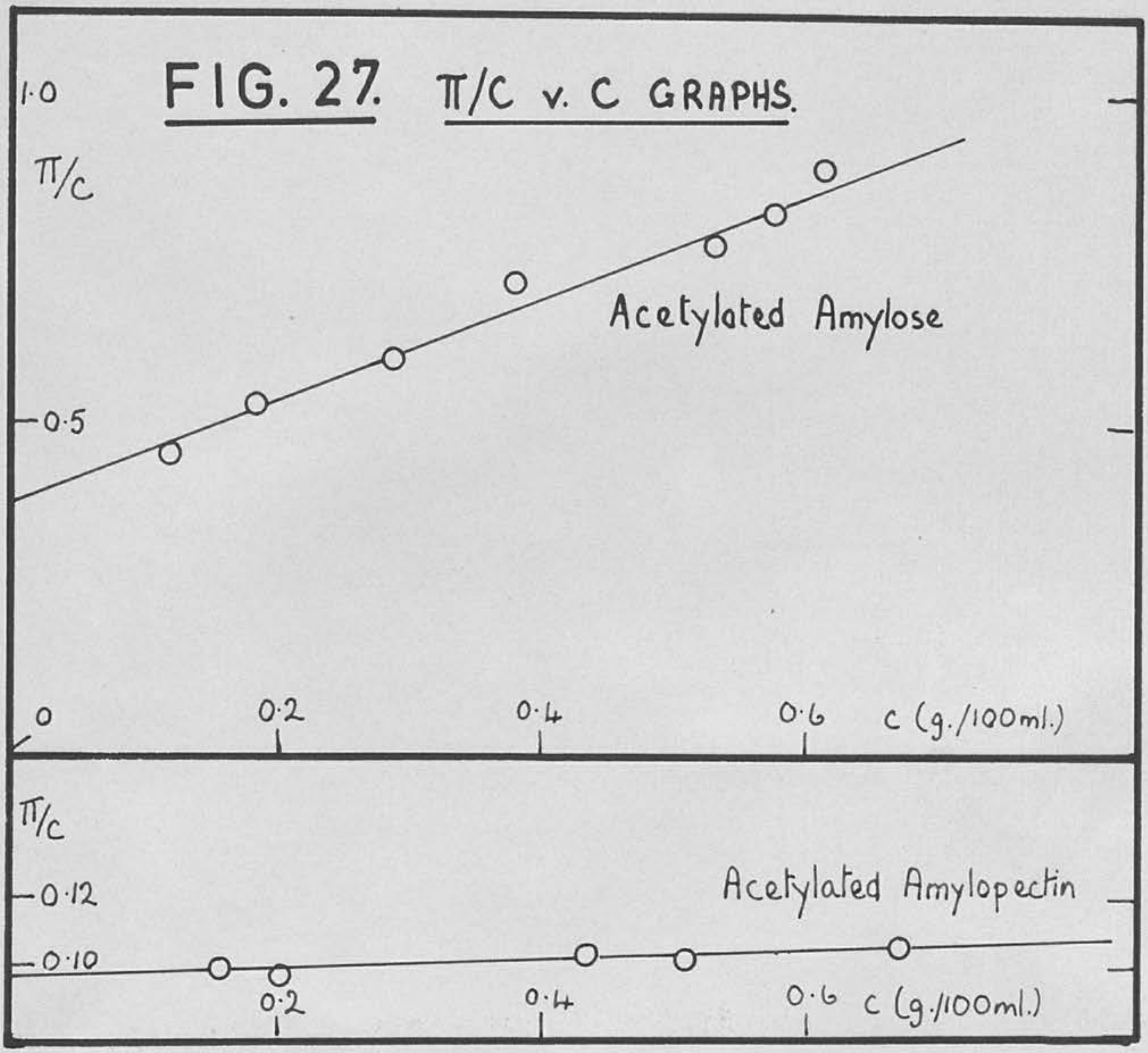


FIG. 27. π/C v. C GRAPHS.
1.0
π/c
Acetylated Amylose
0.5
0
0.2
0.4
0.6
c (g./100ml.)
π/c
Acetylated Amylopectin
0.12
0.10
0.2
0.4
0.6
c (g./100ml.)

against c to be 0·097, whence M_n = 1,800,000.

The density of a 1% solution of the sample in chloroform was 1·4885 g./ml. From this result the correction applied to the observed osmotic pressure was calculated. A most important correction due to the small osmotic pressures.

Acetylated Amylose

Concentration (c g./100 ml.)	Corrected Osmotic Pressure (π cms. solvent)	$\frac{\pi}{c}$
0·6124	0·549	0·900
0·5751	0·472	0·821
0·5316	0·413	0·773
0·3784	0·272	0·719
0·2868	0·172	0·600
0·1823	0·098	0·538
0·1207	0·055	0·456
0	-	0·380

The limiting value of $\frac{\pi}{c}$ as $c \to o$ was found by extrapolation to zero concentration of the graph of $\frac{\pi}{c}$ against c to be 0·380 whence $\bar{M}_n$ = 440,000

The density of a 1% solution of the sample in chloroform was 1·4819 g./ml. From this result the correction applied to the observed osmotic pressure was again calculated.

DISCUSSION

The results for the size of the amylose fractions obtained by various fractionation procedures from potato starch are shown in Table 30. It is apparent that the aqueous leaching procedure of Gilbert and Baum[84] leads to a product (amylose H) having a considerably smaller

D.P. than that obtained by the conventional fractionation techniques (amyloses J and K). The product is in fact, little different from that obtained by aqueous leaching at 70°C (A70).

When the sizes of the products from the conventional fractionation procedures are compared, it can be seen

Table 30

The Size of Amyloses from Various Fractionation Procedures.

Sample	Fractionation Procedure	[η] 1M KOH	[η] of OAc in $CHCl_3$	$\bar{M}_n$	$\overline{D.P.}$
A70	Leaching at 70°C	2.40	5.10	495,000	1,700
A98	Conventional fractionation after A70	-	7.80	1,500,000	5,200
H	Aqueous leaching at 98°C	2.80	5.20	670,000	2,300
J	Conventional fractionation: 2 hr. dispersion	4.60	6.40	888,000	3,100
K	Conventional fractionation: 1 hr. dispersion	5.00	6.80	1,110,000	3,870

that even in a nitrogen atmosphere the amylose product is labile to a limited extent, and that no advantage is gained by increasing the length of time of dispersion of the starch.

The importance of a nitrogen atmosphere for avoiding inadvertent oxidative degradation during fractionation is illustrated in Table 31 where the $\overline{D.P.}$ of the samples prepared under strictly oxygen-free conditions are compared with previous values reported in the literature.

Table 31

Table 31

Molecular Weights of Acetylated Potato Amyloses.

Sample	M.Wt.Method*	Solvent	M.Wt.	$\overline{D.P.}$	Ref.
K	O.P.	$CHCl_3$	888,000	3,100	-
J	O.P.	$CHCl_3$	1,110,000	3,870	-
Gilbert	O.P.	$CHCl_3$	1,100,000	3,800	-
-	O.P.	$C_2H_5NO_2$	326,000	1,130	92
-	O.P.	$CHCl_3$	260,000	930	93
-	S.& D.	MeOAc	69,000	240	94
-	O.P.	$CHCl_3$	245,000	850	95

* O.P. = osmotic pressure; S.& D. = sedimentation and diffusion.

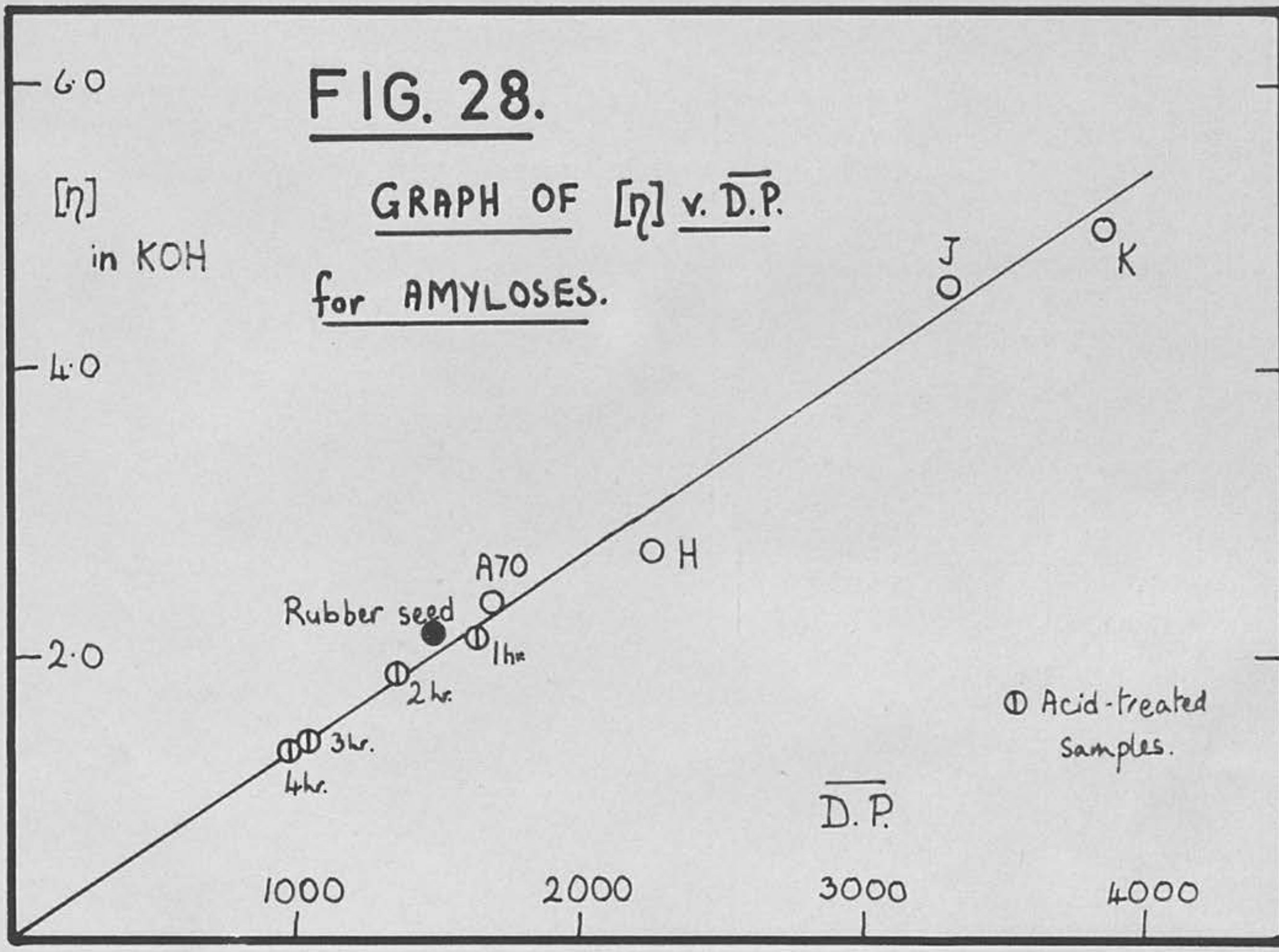
The other samples studied have obviously been degraded, and the $\overline{D.P.}$ of undegraded potato amylose would appear to be of the order of 4000 glucose residues.

The difference between the $\overline{D.P.}$ of A70 and A98 is of the same order as that found previously by Meyer,[89] and suggests that there may well be two distinct molecular weight species of amylose in potato starch.

Measurement of the $\overline{D.P.}$ of a sample of amylose prepared from rubber seeds by Greenwood and Robertson[83] showed it to possess a $\overline{D.P.}$ of about 1500 residues. It was therefore rather smaller than potato amylose. The corresponding amylopectin had a molecular weight of 1,800,000 i.e. a $\overline{D.P.}$ of 6000. This value in conjunction with the average length of unit chain of 23 glucose residues,[83] showed that the amylopectin had a highly branched structure (ca. 260 branches/molecule).

In the case of the amylose samples, the relationship

$\overline{D.P.} = 7{\cdot}4 \times 10^2 \times [\eta]$, where $[\eta]$ is the limiting viscosity number of the free component in alkali was found when values of $[\eta]$ are plotted against the D.P. of the corresponding acetate (derived from osmotic pressure measurements). (See Fig.28). A similar relationship holds for rubber seed amylose.


6.0
FIG. 28.
[η]
in KOH
GRAPH OF [η] v. $\overline{D.P.}$
for AMYLOSES.
J
K
4.0
H
A70
Rubber seed
1hr.
2.0
2hr.
3hr.
4hr.
Acid-treated samples.
$\overline{D.P.}$
1000
2000
3000
4000

SUMMARY.

SUMMARY.

Methods of investigating the size of polysaccharide molecules have been studied. Isothermal distillation has been shown to be suitable for molecular weights of less than 20,000, and for such materials it provides an easy routine method. A new design of ebulliometer for this low molecular weight range has also been evolved. The method of osmometry has been studied in detail. Several types of instrument have been developed. Molecular weights in the range of 20,000-2,000,000 have been determined using this method. Viscosity measurements have been used to provide a convenient method for characterizing the high molecular weight polysaccharides studied.

These physical methods have been applied to several problems. Inulin in Dahlia tubers has been subjected to a graded series of aqueous extractions, and the properties of the resultant fractions examined. The molecular weights of a series of xylans, lichenins, laminarins, and other glucosan samples have been determined by isothermal distillation and osmometry. From these data and by comparison with the results of chemical end-group assay, the molecular structure of these polysaccharides has been determined.

Some fundamental problems in starch chemistry have been tackled. These include the nature of the granular structure of potato starch and estimations of the size

of the components obtained by different fractionation procedures.

The action of acid on potato starch has been studied by fractionating the acid-treated samples and measuring the molecular weights of the components. A preferential degradation of amylose was found, whilst the amylopectin was relatively unaffected. The significance of this with regard to the granular structure is discussed. Several methods involving aqueous leaching as a means of fractionating potato starch have been studied, and shown to involve a molecular weight fractionation of the amylose present. The importance of fractionating under oxygen-free conditions has been demonstrated, and potato starch amylose is shown to possess a $\overline{D.P.}$ of about 4,000 glucose units. For potato amylose, the relationship

$\overline{D.P.} = 7 \cdot 4 \times 10^2 \times [\eta]$ (where $[\eta]$ is the limiting viscosity number of the free component in 1M potassium hydroxide) has been found. Investigations of rubber seed amylose and amylopectin have been carried out.

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1)

Because of a Polysaccharide consists of molecules of diff sizes a M.W. ~~distribution~~ det. results in an average value The value obtained depends on the method used.
(A) If the method depends on colligative properties eg. OP & V.P. then the result is a number average ~~eg.~~ M.W. ~~M.~~ i.e.

$$\bar{M}_n = \frac{\sum_i n_i M_i}{\sum n_i}$$

n_i is no of gram. moles. of M.W. M_i and the summation is taken over all values of i.

Methods dep. not only on conc but also on weight of particle i.e light scattering and ~~or~~ Sedimentation give a weight average defined as

$$\bar{M}_w = \frac{\sum_i n_i M_i^2}{\sum n_i M_i}$$

The M.W. as det. by chemical end group assay is a number average value. For an accurate comparison, therefore, of such chemically based results with those from physico chemical measurements, the latter must also give a number average M.W. In this respect iso thermal distillation is suitable and so will be applied in this work. Such a method does however have limitations. The most important being the range in which it is applicable viz. a M.W. range of 1,000 – 20,000.

Iso-thermal distillation. Many polysaccharides (eg. ½ celluloses fructosans and glucosans have ~~the~~ number average M.Wt's in the range 1000 – 20,000. and so iso-thermal distillation is one of the methods applicable to the det. of molec

3.

weights of such polymers. The method depends on the activity of a solution being less than that of a pure solvent. If a solution is ideal and dilute the difference of activity is equal to the solute mole fraction and also equals the difference in V.P. between solution and solvent. Now a solution in which the solute is assoc. or dissoc. no longer obeys Raoult's law and the activity of such a solution is not ideal. We must therefore assume ~~that~~ all solutions obey Raoult's Law. ∴

$$\frac{P_0 - P}{P_0} = \frac{n}{n+N} = \frac{\frac{W_2}{M_2}}{\frac{W_1}{M_1} + \frac{W_2}{M_2}}$$

P_0 = V.P. of Solvent.
P = V.P. of Solution
$\frac{n}{n+N}$ is Solute mole fraction
W_2 and W_1 are weights of Solute & Solvent.
M_2 and M_1 " M. Wts of " "

Now a Solution of a Polysaccharide with a M.W. of 1000 - 20000 has a detectable V.P. lowering and Paddington (Canad. J. Chem. 33. 447 (1955)) has in fact described an apparatus in which a sensitive mercury manometer measures directly the V.P. of a solvent and polymer solution. Similar apparatus has been tried in this dept. but proved unsuccessful.
Now when a solvent and solution are in a closed system at constant temperature with only their vapour phases connected, isothermal distillation takes place. In such a system solvent vapour distils from

3

The solvent into the solution. Both the total amount of solvent distilling and the rate of the distillation are proportional to the vapour pressure lowering of the solution.

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ONTENTS

ALL, LEO A., and BROWN, D. W.: Use of Deuterium to Investigate Extent of Branching in Polystyrene 513

RANCKEN, M., and SMETS, G.: Polymers and Group Interactions. I. Synthesis of Polyampholytes 521

AWARD, R. N.: Polymerization of Diallyl Phthalate 535

HARLESBY, A.: Effect of Molecular Weight Distribution on Gel Formation by High Energy Radiation 547

ILLER, JR., L. A.: The Reaction of Cellulose with Acetic Acid 555

ASE, SHIGEO: How to Treat Tensile Data of Rubber. III. Least Squares Adjustment... 579

ELLINEK, H. H. G., and NORTHEY, HELEN L.: Adsorption of High Polymers from Solution on to Solids. II. Adsorption of Polystyrene on Charcoal 583

ook Review: Das Makromolekül in Lösung, H. A. Stuart, ed. Reviewed by H. Mark..... 588

EORGIEFF, K. K.: The Relative Inhibitory Effect of Certain Acetylene Polymers on the Rate of Polymerization of Acrylonitrile and Vinyl Acetate (Letter to the Editors).... 589

ROATCH, W. N., and GREENWOOD, C. T.: Observations on Some Possible Errors in Osmometry (Letter to the Editors) 593

uthor Index, Volume XIV 597

ubject Index, Volume XIV 600

olume Title Page i

olume Contents iii

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Observations on Some Possible Errors in Osmometry

Number-average molecular weights ($\bar{M}_n$) obtained from osmotic pressure measurements for a given sample often show variation.[1] In a recent communication, Cleverdon and co-workers[2] have discussed some of the possible causes for the discrepancy between the value of $\bar{M}_n$ found by them and by Hookway and Townsend[3] for a broad fraction of commercial polystyrene. Through the courtesy of Dr. D. Cleverdon we have also been able to examine this sample, and wish to report briefly our observations.

Osmotic pressures of solutions of the polystyrene in chloroform were measured using an instrument previously described[4] (for the loan of which we are indebted to Dr. G. A. Gilbert), in which the diameter of the solvent capillary had been increased to 0.04 cm. The cell constant was then independent of the meniscus level and was reproducible within the setting of the cathetometer (± 0.001 cm.). In addition, one measurement was made in a Fuoss-Mead instrument, modified for complete immersion[5] and fitted with 0.1 cm. diameter capillaries, in which the cell constant was negligible (± 0.001 cm.). The membranes used were of No. 600 gel-cellophane, which had been dehydrated by acetone and then conditioned to solvent. Pressures were measured statically after an initial setting to within -0.1 cm. of the expected value. Although gel-cellophane membranes have been described as truly semipermeable down to $\bar{M}_n \approx 10{,}000$,[6] solute permeation was found to occur in both osmometers, which suggested that the sample was indeed a very broad fraction. The initial rate of decrease in the static pressure head was *ca.* 1.0%/hr. in the Fuoss-Mead instrument, and *ca.* 0.3%/hr. in the other osmometer, and when correcting for this it was assumed legitimate to extrapolate pressures linearly to zero time (0.04% solute permeated into the solvent chamber when a solution containing 1.257 g./100 ml. was left for 72 hr. in the Fuoss-Mead instru-

TABLE I

OSMOTIC PRESSURE MEASUREMENTS FOR POLYSTYRENE SAMPLE IN CHLOROFORM SOLUTION AT 22.5°C.

Concentration (C) in g./100 ml. solution	Osmotic pressure (π) in cm. solvent	π/C
1.257	2.596	2.07[a]
0.956	1.682	1.76
0.766	1.238	1.62
0.487	0.641	1.32
0.443	0.569	1.28
0.358	0.413	1.15
0.270	0.280	1.04
0.165	0.169	1.02
0.079	0.075	0.95
0	—	(0.86)[b]

[a] Measurement in the Fuoss-Mead osmometer.
[b] By method of least squares.

ment). The results, shown in Table I, include corrections for the density of solution.[5] The π/C *vs.* C curve appeared linear, and application of the method of least squares showed that the data were best represented by the equation:

$$\pi/C = 0.861 + 0.952\, C$$

(The standard deviation in π/C was 0.023.)

These figures give a value of 195,000 ± 5,000 for $\bar{M}_n$, which may be compared with 222,500 and 210,000 obtained using denitrated collodion membranes in Fuoss-Mead instruments[2] and 156,500 using polyvinyl alcohol membranes in a Zimm-Myerson instrument.[3] Although the latter values were from measurements in benzene solution, a value of 210,000 has been obtained in chloroform solution by Bawn.[7] Possible causes of discrepancy are (*1*) solute permeation, (*2*) solute adsorption on the membrane, (*3*) membrane "dissymmetry" effects, and (*4*) neglect of an appropriate correction for the density of the solution.

In our measurements, although solute permeation was apparent, a correction has been applied for it. The consistent results from the two osmometers suggested furthermore that this correction was adequate. The effect of solute permeation is far more noticeable in either of the instruments used here than it would be in a Zimm-Myerson osmometer, owing to the different solvent volumes used. There was no evidence of membrane dissymmetry. The effect of adsorption of solute may well vary with the type of membrane used. In our experiments, the effect on the observed osmotic pressures of any random adsorption of solute (*i.e.*, "nonselective" adsorption) on the membrane was eliminated, as all concentrations were measured (in duplicate) *after* each determination using the method previously described.[5] Proof of the absence of any preferential adsorption of either low or high molecular weight solute (*i.e.*, "selective" absorption) is difficult to obtain, but it is thought that such effects were not occurring as (*1*) consistent osmotic pressures were observed independently of the order in which solutions of varying concentrations were measured, and (*2*) in an additional experiment, there was no change in the observed osmotic pressure when the osmometer was filled successively with two solutions of the same concentration (*i.e.*, π = 0.420 and 0.419 cm. chloroform, respectively). Both "selective" and "nonselective" adsorption could have influenced the other determinations carried out on this sample. Similarly, density corrections are often extremely important when high molecular weight substances are being examined, particularly when comparisons are made between results from different osmometers: although negligible in the case of the Zimm-Myerson instrument, they are considerable in the Fuoss-Mead type. For example, if Cleverdon's result of 222,500 is corrected on the basis of an average height of solution column of 20 cm., the value of $\bar{M}_n$ is 215,000. Bawn's results may require a correction of the same order.

Thus, although we have attempted to account for most of the possible sources of error, our result for $\bar{M}_n$ is 10% lower than Cleverdon's corrected value and 20% higher than Hookway's value. Apart from the unpredictable effect of "selective" adsorption occurring in measurements using either denitrated collodion or polyvinyl alcohol membranes, the most likely reason for the discrepancy is the low molecular weight "tail" escaping notice in the measurements giving the higher values, as Hookway claims that polyvinyl alcohol membranes are semipermeable down to $\bar{M}_n \approx 2000$.[3] Low molecular material was certainly present in the sample as shown in our measurements, although it was not apparent in those of Cleverdon.[2] It appears likely that the nonideal semipermeability of the membranes could account for the discrepancy. If this is so, then extrapolations applied for solute permeation in our measurements must be incorrect. However, it is difficult to reconcile this with the observed π *vs.* time curves unless there is an instantaneous diffusion of very small material in the few minutes required to adjust the levels, etc., before measurements could be taken.[8] It may also be noted that Staverman[9] believes that this method of correcting for solute diffusion is inadequate.

Further investigations are clearly necessary, and probably comparisons should be made using these different membranes and osmometers on a sharply fractionated high molecular weight polymer.

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